

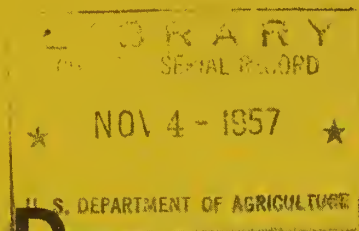
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NEW



IMPROVED COTTONSEED MEALS

for feeding poultry and swine

1944 - 55

AN ANNOTATED BIBLIOGRAPHY

... of publications by participants in
a cooperative research program on
improving cottonseed meal through
processing conditions

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE
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UNITED STATES DEPARTMENT OF AGRICULTURE
 AGRICULTURAL RESEARCH SERVICE
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IMPROVED COTTONSEED MEALS FOR FEEDING POULTRY AND SWINE, 1944-1955 AN ANNOTATED BIBLIOGRAPHY

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SOURCES CONSULTED

Bibliography of Agriculture, Volumes 4 to 19, incl., 1944-55

Chemical Abstracts, Volumes 38 to 49, incl., 1944-55

Biological Abstracts, Volumes 18 to 29, incl., 1944-55

List of Publications and Patents. For the years 1944 to 1955, by the Southern Utilization Research Branch, Agricultural Research Service, U.S.D.A.

The references are divided into six sections, according to the major subject of the items. They are arranged chronologically in each section.

As a guide to the user, cross references to other related articles listed elsewhere in the bibliography appear at the end of each section.

*Deceased.

INTRODUCTION

By

Aaron M. Altschul
Southern Regional Research Laboratory

Cottonseed meal traditionally has been used in the feeding of ruminant animals with only restricted quantities used in feeds intended for nonruminants such as poultry and swine. But, as a result of a broad cooperative research program in which this Laboratory participated, cottonseed meal no longer can be said to be limited exclusively to ruminant feeds. In theory and in practice, there is every indication that cottonseed meal is now a significant factor in poultry and swine feeding.

Substantial quantities of cottonseed meal (estimated to be approximately 250,000 tons in 1954) already have been put to use for nonruminants with the prospect that larger quantities will get into such feeds as the economy demands.

This accomplishment was not the work of one person or a group of people but the result of a cooperative effort involving processors, chemists, and nutritionists: commercial mills, nutrition laboratories in commercial and Federal and State institutions, and chemical laboratories in these various institutions. Over 150 publications have evolved as a result of this effort.

The purpose of this review is to bring together for ready reference the numerous publications which have appeared as part of this effort. Each of these publications is noted and abstracted.

It was difficult to establish a definitive basis for selection of the publications by cooperating groups to be included in this reference work. No doubt some publications are included that do not belong on the list and others may be left out which should have been included. We hope that the authors of such papers will treat these errors of commission or omission in a sympathetic manner, fully realizing the difficulties attendant in selecting papers for this abstract bibliography. The basis for inclusion of an author's work in this bibliography was that he participated in the overall program either by attending meetings held at the Southern Regional Research Laboratory to review the progress or by utilizing samples furnished by the Southern Laboratory in his nutritional evaluations. There were some who did not fit clearly in either of these categories. In building this cooperative program around the activities at the Southern Regional Research Laboratory or on

samples furnished by the Southern Laboratory, it is not our intention to assign to this Laboratory a central role in this program. Such a role cannot be assigned by any contemporaries of a research program; it is merely a point of departure and to us, one with which we are most familiar. Whether one group or individual provided leadership in this program is not really so important as that the program did exist and did indeed succeed.

The publications listed in this bibliography cover the period beginning with January 1944. There has been extensive prior research in this field by many competent investigators who provided a much needed basis for the work that followed. Much of this is reviewed in Cottonseed and Cottonseed Products, their Chemistry and Chemical Technology (New York, Interscience Publishers, 1948).

The actual chronology of events as they took place at the Southern Regional Research Laboratory is somewhat as follows:

January 1944: First paper on pigments of cottonseed was published by Dr. Charlotte Boatner. This led to extensive research on the pigment glands of cottonseed and their properties which led in turn to experiments in mills of the Southern Texas Cotton Oil Company on effect of various conditions of cooking on the properties of the oil.

April 1945: Research was begun in the pilot plant on a method for producing pigment glands and gland-free meal from cottonseed. As a result of this work, sizable quantities of such glands and meal were made available for experimental purposes in various phases of nutritional and pharmacological research.

August 1947: Dr. Paul E. Cannon, University of Chicago, began tests on specially processed meals and gland-free meal. His first findings, indicating considerable variation in the nutritive value of the meals for rats, were reported in a letter to this Laboratory in December.

December 1948: The complete findings of Dr. Cannon were presented to a group of Department scientists at Beltsville at a meeting called at the suggestion of the Southern Regional Research Laboratory. It was agreed to reopen the question of the nutritive value of cottonseed meal, which had been considered hitherto as suitable only in restricted quantities for feeding to nonruminants, in a cooperative program between the Southern Regional Research Laboratory and nutritionists of the United States Department of Agriculture.

November 1949: The first of a series of papers describing rapid methods for the determination of free and bound gossypol was published. These methods had been adopted as tentative by the American Oil Chemists' Society at the 1949 Spring Meeting.

May 1950: A conference was held with Mr. A. L. Ward, Director of Educational Service of the National Cottonseed Products Association, with the result that the cooperative effort was broadened to include this service as well as many of the State Experiment Stations.

November 1950: The Educational Service of the NCPA and SRRL jointly called the first conference on the effect of processing on the nutritive value of cottonseed meal. It was attended by practically all of the workers on cottonseed meal in State and Federal agencies as well as important industrial workers.

November 1951: The Second Conference on Cottonseed Processing as Related to Nutritive Value was held at the Southern Regional Research Laboratory.

July 1952: An interim conference on processing as related to nutritive value was held in Dallas, called jointly by the Educational Service of the NCPA and SRRL.

October 1, 1952: Miss Edith Jensen, Research Fellow for NCPA, was transferred to the program on cottonseed meal, with particular emphasis on developing chemical methods for estimating nutritive value.

November 1953: The Third Conference on Cottonseed Processing as Related to the Nutritive Value was held at Southern Regional Research Laboratory. At this conference it was possible to pass the following resolutions:

- A. Results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean meal alone, when the cottonseed meal has 0.04% or less of free gossypol and 75% or more of nitrogen solubility in 0.02N NaOH solution.

Nutrition investigators representing industrial, federal, and state organizations are invited to conduct experiments based on this statement using cottonseed meal of known processing history and chemical characteristics and soybean meal of high quality.

- B. Preliminary indications are, insofar as free gossypol is concerned, that cottonseed meals having 0.04% or less of free gossypol can be fed in unrestricted proportion in balanced diets for chicks, broilers, and swine.

October 9, 1954: A feature article in the Cotton Gin and Oil Mill Press indicated that cottonseed meal was now being used in nonruminant feeds and in mixed feeds generally. The following is quoted from the opening paragraph: "Money from a new source has flowed into the pockets of cottonseed crushers during the past 12 months. No one has an exact estimate as to the total figure but it is large enough to attract attention throughout the industry."

May 1955: Upon the recommendation of its research committee, of which Harry L. Craig was then chairman, the National Cottonseed Products Association awarded a second fellowship to the Southern Regional Research Laboratory for the purpose of facilitating nutritional investigations of current commercial production of cottonseed meal.

November 16, 1955: The subcommittee on meal of the Research Committee of the National Cottonseed Products Association, with Harold L. Wilcke as chairman, met at the Southern Regional Research Laboratory to plan the type of nutritional evaluations to be conducted on commercial cottonseed meals.

April 1956: Approximately 60 tons of mixed diets containing nine commercial cottonseed meals, alone or in mixtures with soybean meal (28 different diets), were distributed to nutrition investigators for testing on poultry. Samples of the meals were distributed to another seven investigators for studies on other animals.

It is obvious that this is an active continuing program. Members of the staff of the Southern Laboratory consider it a privilege to participate in this program and to enjoy the cooperation of the many scientists and industrialists who are involved.

The status of the development might be summarized as follows:

1. Cottonseed meal enjoys new status as a feed that can be used for nonruminants; it has been used extensively in mixed feeds with satisfactory results. Cottonseed meal is assured a place in mixed feeds; the amounts used will depend upon relative costs of meals and on the quality of the cottonseed meal

produced. Cottonseed processors are now aware that processing affects the suitability of their product in feeds for poultry and swine. Many of them are conducting tests routinely on their production to ascertain their suitability for such markets, and quite a few are striving conscientiously to broaden the utilization of their meals in nonruminant feeds.

2. The production of the improved meals has been achieved through an understanding of the fundamental requirements of such meals and through improved practice. It was realized that a good meal can be produced only if it contains low free-gossypol content, achieved without damaging the meals by heat treatments. Usually the low free-gossypol content could be achieved by a process involving screw-pressing, either direct or as a prepress operation. The use of any other method of removing the oil required specialized treatment to accomplish the same purpose. Direct solvent extraction can be used under circumstances involving control of the cooking, or chemical treatment of the oil-free meal. Not all screw-pressing is suitable because in many instances the energy involved in pushing the material through the press is so great as to create considerable heat damage. Nevertheless a sizable proportion of the present commercial production is suitable for most uses in nonruminant feeds.
3. Some criteria exist for judging the suitability of meals in nonruminant feeds. It is generally agreed that one of the prerequisites is a low free-gossypol content of the order of magnitude of 0.04%. It is also agreed that there must be minimum heat damage which can be measured in a crude way by the solubility of the meal in dilute alkali (70 - 75% solubility in 0.02N alkali). Admittedly neither of these criteria are the last word on the subject and do not unequivocally predict the feeding value of every meal that is produced. They have the advantage of alerting the industry to the need for care in production of meals and to the desirability of passing at least crude criteria.
4. There exists a research program in many institutions on improving our information on the properties of the cottonseed meals and there exists a history of a large-scale cooperative effort that makes it easier to continue such type of cooperation in the future.

While the achievements have been profound in that they have brought about a change in the character of the industry, their most important effect has been to open up the question of cottonseed processing to serious scrutiny and to re-evaluation. The process of the extraction of oil from cottonseed, as for other oilseeds, developed primarily as a method for removing the greatest amount of oil with a little regard to the quality of the residue. In the early days of cottonseed processing, this was a sound approach because the value of the oil was so much greater economically than that of the meal. Only in recent years has the value of the meal approached that of the oil. In 1955 the oil provided about 58 percent of the value of the products of the seed, whereas the meal provided 31 percent of that value. Although the meal provided less income than the oil, the economic value of the meal was close enough so that it became desirable to consider meal quality as one of the factors in the processing of cottonseed.

A compromise must be effected now between the yield of oil, quality of oil, quality of meal, and throughput to yield the best economical benefit from cottonseed processing. This compromise is not necessarily the best one because there does not exist a process for cottonseed which will achieve the best of these objectives without reflection on one or the other of the components of the seed.

This indeed then should be the objective of future research on cottonseed processing: to obtain information which would allow for the development of a method of processing of cottonseed which will take into consideration the unique properties of the seed and provide for the best quality and yield of both oil and meal. In order to accomplish this, it is necessary to obtain much more information about the reactions that take place during cooking of cottonseed and during processing. More information is needed on the reactions of gossypol, the most important minor component, as they affect the quality of the meal and oil. It is known that gossypol in oil causes discoloration which lowers its quality. It is known that high free-gossypol in the meal is toxic and renders it unsuitable for growing poultry and swine. Even low quantities of free gossypol make the meal unsuitable for use in an egg-laying ration. There is even some question about the bound gossypol and some insist that bound gossypol is achieved at the expense of lowering the nutritive value of the meal. More information on these questions is needed in order to provide the basis for a suitable process. When it is known what happens to the proteins during processing, it will be possible to devise better methods of predicting the nutritive value of meals by their chemical analyses.

It may be expected in the years to come, if this abstract bibliography is revised to bring it up to date, that much more progress will be indicated and, perhaps, the cottonseed industry will have succeeded in developing a process which brings out the greatest potentials for the use of its products, oil and meal.

I. PROGRAM

Objectives and Scope

1. SOUTHERN UTILIZATION RESEARCH LABORATORY. ANNUAL COOPERATIVE COTTONSEED OIL MILLS CONFERENCES, JOURNAL OF PROCEEDINGS. First to the fifth meetings held at the Southern Regional Research Laboratory, New Orleans, La., for the years 1949 to 1954, respectively; the sixth meeting (soybean and cottonseed combined) held in Lubbock, Tex., in 1955. (Processed.)

Working conferences of cooperative oil mill managers, Farmer Cooperative Service, and research workers of SRRL.

Research programs described include cottonseed processing, as it affects the quality of oil and meal; survey of composition of various meals; and nutritive value of meal.

2. DECHARY, J. M., and ALTSCHUL, A. M. RESEARCH ON EFFECT OF PROCESSING ON THE NUTRITIONAL VALUE OF COTTONSEED MEAL. Oil Mill Gazetteer 54(2): 13-15. 1949.

In cooperation with the cottonseed industry and nutritionists, SRRL has inaugurated research on improving the nutritional value of cottonseed meal by developing new methods or improving present methods of processing. The value of cottonseed meal as a feed depends on quality of protein; also on presence or absence of materials that interfere with growth of animals. These materials are concentrated in glands--consisting of gossypol and gossypol-like compounds. They can be bound to the proteins of the meal during processing so that they no longer interfere with the food value of the meal. Once the proper conditions for processing are defined and methods for production control are developed, it should be possible for the industry to produce a more uniform and far superior cottonseed meal.

3. SOUTHERN REGIONAL RESEARCH LABORATORY PROCEEDINGS OF A RESEARCH CONFERENCE ON PROCESSING AS RELATED TO COTTONSEED MEAL NUTRITION, 68 pp. 1950. PROCEEDINGS, SECOND CONFERENCE ON COTTONSEED PROCESSING AS RELATED TO THE NUTRITIVE VALUE OF THE MEAL, 107 pp. 1951. PROCEEDINGS OF THIRD CONFERENCE ON PROCESSING AS RELATED TO NUTRITIVE VALUE OF COTTONSEED MEAL, 59 pp. 1953. (Processed.)

Representatives of industry, state universities, agricultural experiment stations, and government officials

interested in cottonseed meal production and utilization met with members of the Southern Regional Research Laboratory to report and evaluate recent cooperative investigations on conditions employed in production of cottonseed meal of improved quality and significant results in the utilization of these cottonseed meals in animal feeding studies.

Among the recommendations of the third conference (page 48, 1953) is a "Statement of Policy Regarding Use of Cottonseed Meal in Feedstuffs" as follows:

"A. Results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean alone, when the cottonseed meal used has 0.04 percent or less of free gossypol and 75 percent or more of nitrogen solubility in 0.02N NaOH solution.

"Nutrition investigators representing industrial, federal, and state organizations are invited to conduct experiments based on this statement using cottonseed meal of known processing history and chemical characteristics and soybean meal of high quality.

"B. Preliminary indications are, insofar as free gossypol is concerned, that cottonseed meals having 0.04 percent or less of free gossypol can be fed in unrestricted proportion in balanced diets for chicks, broilers, and swine."

4. ALTSCHUL, A. M.

RESEARCH ON COTTONSEED STORAGE AND PROCESSING AT THE SOUTHERN REGIONAL RESEARCH LABORATORY. Cotton Gin and Oil Mill Press 52(16): 24, 26, 28-30, 32; Cotton Digest 23(46): 6-7, 42-43, 46; Cotton Res. Congress 12: 45-52. 1951.

In cooperative research with the Educational Service of the National Cottonseed Products Association, SRRL has provided nutrition investigators with cottonseed meals of known processing history. The investigators found that experimentally made screw-pressed meals can be fed to hogs in a concentration up to 43 percent

of the total diet and to chicks in a concentration up to 70 percent. Lower temperatures of cooking in screw press operations resulted in meals with higher nutritive value and in some meals which did not cause yolk discoloration in stored eggs, even when fed at levels of 20 to 25 percent of the diet.

5. HARPER, G. A.

SWINE AND POULTRY OFFER COTTONSEED MEAL IMPORTANT NEW MARKETS. Cotton Gin and Oil Mill Press 52(20): 8, 10. 1951.

The nutritional research program of National Cottonseed Products Association is presented and several nutrition scientists who have received honorary awards and recognition are cited.

The importance of continuing research to improve on what already has been accomplished on processing cottonseed meals and incorporation of cottonseed meals in feeds is discussed from an economic standpoint. The necessity of utilization of cottonseed meal in feed supplements in the current increased competition between the many feed supplements is stressed.

The intensified research program underway in which industries, nutrition scientists, state and education experiment stations, National Cottonseed Products Association, and Southern Regional Research Laboratory have cooperated, has resulted in the production of cottonseed meals which can be used in unlimited amounts for swine and poultry. These cottonseed meals compare favorably with other protein concentrates which are now being used more extensively than cottonseed meals in rations fed poultry and hogs.

6. ALTSCHUL, A. M.

CHEMICAL PROPERTIES AND NUTRITIVE VALUE OF COTTONSEED MEAL AS RELATED TO CONDITIONS OF PROCESSING. Natl. Cottonseed Products Assoc. Off. Proc. 55: 32-34, 36. 1951.

Cottonseed meal competes with other sources of protein, such as soybean meal, for feed markets--in addition, with urea for use in ruminant feeds. The value of cottonseed meal as a feed depends on type of animal; amino acid content of the protein; damage to protein that took place during processing; and presence of materials that would interfere with growth and food utilization. Research has shown that cottonseed meal is a variable nutritional material and that conditions of cooking prior to removal of oil in the screw press greatly affect protein value.

7. SOUTHERN REGIONAL RESEARCH LABORATORY
PROCEEDINGS, COTTONSEED PROCESSING CLINIC.
First held April 14-15, 1952, 59 pp.; second held
March 9-10, 1953, 98 pp.; third held Feb. 15-16,
1954, 62 pp.; fourth held Feb. 7-8, 1955, 69 pp.

Working conferences were held in cooperation with the Valley Oilseed Processors Association. SRRL staff members reported on the research program on cottonseed and cottonseed products with particular emphasis on the relation and application of research results to the practical operating problems of the industrial processor.

Such topics as better protein feeds from cottonseed, research on conditions of processing cottonseed to improve quality of oil and meal, status of research on improving nutritive value of meal were discussed by A. M. Altschul.

8. ALTSCHUL, A. M., and BARINGER, K. L.
BETTER PRODUCTS FROM COTTONSEED THROUGH
RESEARCH. Cotton Trade Jour. 34(50): 6; (51): 6;
(52): 6. Dec. 5, 12, and 19, 1952.

Aims and results under a cooperative research program between this Laboratory and other Federal agencies, State Experiment Stations, the National Cottonseed Products Association, and cottonseed oil mills, whose objective is to improve the nutritive value of cottonseed meal without impairing oil quality, are described.

9. ALTSCHUL, A. M., and THURBER, F. H.
PROCESSING OF COTTONSEED. I. REVIEW OF
RESEARCH TO IMPROVE NUTRITIVE VALUE OF
MEAL AND COLOR OF OIL. II. PRELIMINARY
REPORT OF RESULTS OF LABORATORY RE-
SEARCH. Cotton Gin and Oil Mill Press 54(23):
26, 68-71. 1953.

Problems involved in improving the quality of cottonseed meal and oil are reviewed. Some commercial meals now available are more suitable for feeding to growing swine and poultry than the average meals produced. Surveys now in progress will provide a comparison between prepressed solvent-extracted and other types of cottonseed meals.

Also reviewed is the research underway to aid in solving these problems. Preliminary laboratory

research results are reported on effects of different conditions of cooking on nutritive value of meal as measured by the growth rate of young chickens; on methods for the removal of the pigments responsible for the color in cottonseed oil; on the nature of bound gossypol; on the initial content of gossypol in the seed; on reactions between gossypol and other components of the seed during cooking that affect nutritive value. Results of these experiments will act as a basis for developing a chemical measure which will be more reliable and will correlate more closely with actual nutritional measurements.

10. GRAU, C. R.

COTTONSEED MEAL--PROBLEMS AND PROMISE.
Natl. Cottonseed Products Assoc. Proc. 57: 36-37.
1953.

An increase in the use of cottonseed meal in poultry and swine rations has developed as a result of recent research.

Three important accomplishments among the many obtained through the research program are: 1) The ability now to manufacture meals--by any of the procedures--which can be used in unlimited quantities for growth production in swine and poultry rations. 2) Protein value of cottonseed meal is better than once was believed. Cottonseed protein can supply very effectively all the needed amino acids, the components of the protein that are required by poultry and swine for normal growth. 3) Along with the many analytical methods for determining the chemical characteristics of meals, a good laboratory method for estimating the protein quality of cottonseed meal is being developed by Texas A. & M. College.

Future research is still needed for the treatment or production of meals that can be fed in unlimited quantities to poultry for egg production.

11. GILES, W. L.

NEW USE OF COTTONSEED MEAL AND OIL. Oil
Mill Gazetteer 58(11): 73-74. 1954.

Reference is made to the work of Dr. Altschul and his staff in finding new products to extend the use of cottonseed. By employing new processing methods, meals with very low percentages of free gossypol can be produced; thus, creating new markets for meal in swine and chick feeds.

12. WARD, A. L.

PROTEIN PROBLEM. Cotton Gin and Oil Mill Press 55(7): 53-55; Oil Mill Gazetteer 58(11): 22-24. 1954.

Address at the Phoenix meeting of West Coast Division of International Oil Mill Superintendents.

After reviewing briefly the history of cottonseed meal, the author spoke on NCPA's sponsorship of a broad research program in processing methods, paying tribute to the Southern Regional Research Laboratory's leadership in the research.

13. JENSEN, E. A., and ALTSCHUL, A. M.

RESEARCH ON COTTONSEED MEALS. Cotton Gin and Oil Mill Press 55(11): 16, 18, 50-51. 1954.

It has been shown experimentally that high-quality cottonseed meals can be produced at commercial mills and that some of the processes now in use are capable of producing such top-quality meals. Processors are unable, however, except by costly, time-consuming animal feeding tests to estimate the nutritive value of a meal. Efforts toward the development of a chemical measure to estimate nutritive value include experiments to determine: 1) on commercial samples the effect of prepress-solvent extraction methods of processing on the chemical and nutritive properties of cottonseed meals; 2) on laboratory-scale samples the effects of variations in cooking conditions on the quality of cottonseed meal and oil; 3) the nature of "bound" gossypol; 4) the properties of the pigments of cottonseed oil.

To develop a chemical measure of nutritive value, it was first necessary to understand how heat alone affects the chemical and nutritive properties of cottonseed meal. Previous work had indicated that heat alone is effective in reducing the nutritive value of cottonseed protein. This work served as the basis for the present study, which sought to compare chemical and nutritional measurements on autoclaved meals. As the first step, an oil-free meal undamaged by heat was chosen as the standard. This meal of low gossypol content and high nutritive value had been prepared by successive extractions with hexane and butanone.

14. WALLACE, H. D.

RESULTS OF LOW-GOSSYPOL COTTONSEED MEAL RESEARCH IS DISCUSSED. Florida Cattleman 19(2): 44. 1954.

The limitation of using cottonseed meal in diets for ruminants is due to gossypol content. At the Southern

Regional Research Laboratory and plants in the southern area, research is being conducted on the changes in and effect of processing conditions. The result has been production of low-gossypol content cottonseed meals.

The experimental low-gossypol cottonseed meals have proved satisfactory in swine feeding, even when fed as high as 30 percent of the ration.

15. WARD, A. L.

COTTONSEED MEAL. Amer. Feed & Grain 38(10): 49-50, 66-67. 1954.

Mr. Ward discusses the increased production and utilization of improved cottonseed meals through research.

Recent production of cottonseed meals with free gossypol contents of 0.04 percent or less, and nitrogen solubility contents of 75 percent or more (in 0.02N sodium hydroxide solution), has extended the utilization of meals to poultry and swine rations, in addition to rations fed cattle and sheep.

Supplementations of swine and poultry rations with cottonseed plus soybean meal have shown a better balance of amino acids, than either meal supplemented singly.

16. WARD, A. L.

COTTONSEED MEAL AND CAKE: RESEARCH WITH RESULTS. Assoc. Amer. Feed Control Off. Off. Proc. 1954: 67-71. 1954.

Cottonseed supplies the largest tonnage of all the oilseeds in the crushing industry.

Work conducted by federal and state experiment stations have added to the prestige of cottonseed. While cottonseed meal can be used as a supplement to rations for cattle and sheep without restrictions, there are imposed limitations to its use in poultry and swine rations. This limitation spurred efforts for additional research for the production of high quality, improved cottonseed meal, which could be used in unlimited quantities for poultry and swine.

At recent seminars, favorable reports have been presented on the progress of the research program. Cottonseed meals prepared by the various methods of processing have been obtained and incorporated into swine and poultry diets at high levels without toxic or growth-depressing effects.

Hydraulic processed meals, having free gossypol contents of 0.04 to 0.1 percent, have been fed from 9 to 10 percent of pig rations without adverse effects--and at higher levels for chicks. However, egg discoloration upon storage was obtained with these meals.

Screw-pressed meals, usually containing 0.04 percent free gossypol or less, have been fed to chicks in amounts up to 70 percent of the ration with safety, and to pigs in amounts of 31, 40, and 43 percent of the total ration.

Prepressed solvent-extracted cottonseed meals have free gossypol contents similar to those of screw-pressed meals. Current feeding tests appear to indicate a high nutritive value for these meals. Solvent-extracted meals exhibit various amounts of gossypol content and nutritive values depending upon solvent and processing conditions employed.

Additional work on pilot plant and commercially processed cottonseed meals, on a cooperative research program, is being conducted to determine the optimum processing conditions for obtaining cottonseed meal of the highest possible nutritive value.

17. JENSEN, E. A.

RESEARCH ON COTTONSEED MEALS. Cotton Gin and Oil Mill Press 56(12): 50-53. 1955.

The work of the NCPA Fellow at the Southern Laboratory has been directed toward the development of a chemical measure of nutritive value of cottonseed meal. The determination of nitrogen solubility in 0.02N sodium hydroxide, suggested as an indication of nutritional quality, can be considered only as an intermediary in the solution of this problem. It seems likely that an understanding of the individual factors involved in nitrogen solubility, such as solubility in various solvents, and effects on individual amino acids will be necessary to arrive at an improved chemical measure of the nutritive value of all types of cottonseed meal.

18. HOPPER, T. H.

SIGNIFICANCE OF NEWER LABORATORY METHODS FOR ANALYSIS OF COTTONSEED PRODUCTS. Oil Mill Gazetteer 60(3): 28-29. 1955.

The long use of laboratory methods for the control of processing and the evaluation of products by the cottonseed industry is cited. The use of new methods for analysis is discussed briefly. Those mentioned include free and total gossypol, nitrogen (protein) solubility, and an improved method for determining residual lint.

19. ALTSCHUL, A. M.

STATUS OF RESEARCH ON CHEMICAL METHODS
OF MEASURING NUTRITIVE VALUE OF COTTON-
SEED MEAL. Oil Mill Gazetteer 60(5): 10-12. 1955.

The place of cottonseed meal as a protein supplement for ruminants and nonruminants (poultry and swine) is discussed briefly. Free gossypol is generally accepted as a reliable index to toxicity of cottonseed meal in the feeding of poultry and swine, and the effects of various methods of processing on the free gossypol content are outlined. Nitrogen solubility tests are widely used to measure nutritive value. High temperatures during processing tend to reduce nitrogen solubility. The paper is a review of the status and prospects of research on cottonseed as a protein supplement for feeding of non-ruminants.

SEE ALSO: 105, 111, 132

II. PIGMENTS

Isolation and identification, effect of storage conditions, reaction of pigments, oral toxicity, detoxification

20. BOATNER, C. H.

THE PIGMENTS OF COTTONSEED. Oil and Soap 21(1): 10-15. 1944.

The presence of dark, unstable pigments in cottonseed is a problem in the use of cottonseed as a source of oil and protein. This investigation was conducted to establish a chemical basis for the color changes occurring in cottonseed during storage, with the idea that this knowledge would help in controlling the pigmentation to avoid undesirable color changes. Although the first scientific study of cottonseed dates back to 1861, only one pigment--gossypol--had been heretofore isolated and its chemical properties determined. It is known that gossypol is a hexahydroxy-binaphthalene compound having as substituents two carbonyl groups and two isopropyl groups. In this investigation a method for the purification of gossypol was developed and the existence of at least three pigments was demonstrated; in addition to gossypol a red pigment, shown to be an oxidation product of gossypol; a yellow oil-soluble pigment, not extractable by alkali, shown to be responsible for some, if not all, of the color of alkali--refined cottonseed oil; and a purple, unstable pigment called gossypurpurin. Some of the properties of the three pigments are reported. A colorimetric test for extractable gossypol is described.

21. BOATNER, C. H., CARAVELLA, M., and SAMUELS, C. S.
AN ORANGE-COLORED PIGMENT OF COTTONSEED.
Amer. Chem. Soc. Jour. 66(5): 838-839. 1944.

A procedure for isolating an orange-colored pigment, based on reextraction of an ethereal extract of cottonseed with a dilute aqueous solution of sodium hydroxide containing sodium dithionate, is described. The pigment differs markedly from gossypol. Its nature, its properties, and reactions have been studied.

22. BOATNER, C. H., and HALL, C. M.

THE PIGMENT GLANDS OF COTTONSEED. I. BEHAVIOR OF THE GLANDS TOWARD ORGANIC SOLVENTS. Oil and Soap 23(4): 123-128. 1946.

Fundamental properties of the pigment glands of cottonseed were investigated. Cottonseed pigments differ

markedly from other plant pigments not only in their chemical nature but in their distribution in the seed. They occur in walled-off cavities or glands many times larger than the surrounding parenchyma cells. The amount of pigmented material extracted from the seed is a function of the type of solvent used. The effectiveness of a given solvent for extracting pigments is determined by its solvent power for the pigments and also by its ability to attack the pigment glands and the extent to which these glands are exposed to its action, which in turn depends on the degree of subdivision of the seed being extracted. Properties of the pigment glands have been utilized for the development of a method for mechanically fractionating cottonseed into pigment glands, embryo tissue (meal), and hull tissue. Finely divided cottonseed is treated with mixtures of inert liquids having densities intermediate between those of the seed parts being separated. This fractionation method is applicable to prepare pigment glands and pigment-free oil and meal from flaked cottonseed and to separate glands from defatted cottonseed.

23. BOATNER, C. H., HALL, C. M., ROLLINS, M. L., and CASTILLON, L. E.

PIGMENT GLANDS OF COTTONSEED. II. NATURE AND PROPERTIES OF GLAND WALLS. Bot. Gaz. 108(4): 484-494. 1947.

The existence of a highly resistant gland wall, rather than membrane, is proposed to explain the anomaly of the relative stability of reactive polyphenolic pigments in cottonseed. Structure and chemical composition of gland wall and its behavior towards various liquids were investigated. The rigid, thick wall-enclosing a gelatinous suspension of the pigments, consists of from 5 to 8 irregularly shaped, curved plates, composed of cellulose apparently impregnated with pectin, hemicellulose, and other unidentified uronic acid derivatives. A layer of cutin surrounds the glands. Glands proved resistant to the action of most reagents except water and polar organic liquids of low molecular weight. The sensitivity of the glands to moisture is proposed to explain various changes during storage of moist cottonseed.

24. BOATNER, C. H., HALL, C. M., O'CONNOR, R. T., and CASTILLON, L. E.

PIGMENT GLANDS OF COTTONSEED. III. DISTRIBUTION AND SOME PROPERTIES OF COTTONSEED PIGMENTS. Bot. Gaz. 109(2): 108-120. 1947.

This investigation was conducted to determine the extent to which pigments of cottonseed occur inside and outside

glands in the seeds; and to investigate the extraction of pigments during extraction of oil by means of different solvents. Methods for the extraction and estimation of pigments of cottonseed have been developed on the basis of the information obtained.

25. BOATNER, C. H., SAMUELS, C. S., HALL, C.M., and CURET, M. C.

THE PIGMENTS OF COTTONSEED. II. GOSSYCAERULIN, A BLUE QUINONOID GOSSYPOL DERIVATIVE OCCURRING IN COOKED COTTONSEED. Amer. Chem. Soc. Jour. 69(3): 668-672. 1947.

A blue pigment, gossycaerulin, has been prepared by heating acidified alcoholic solutions of gossypol, and reaction conditions which favor the formation of this pigment have been determined. Purified gossycaerulin is a polar, alpha hydroxy, quinonoid, oxidation product of gossypol. It exhibits a minimum blue color having an absorption maximum at $605\text{ m}\mu$ in acid solution and is reversibly convertible into a nonpolar, yellow, neutral form. A yellow pigment has been detected as a byproduct during the conversion of gossypol to gossycaerulin. A purple compound resulting from the reaction of gossycaerulin with sulfuric acid exhibits the reactions of a quinonoid compound. Gossycaerulin has been detected in cooked cottonseed. The amount formed increases as gossypol content and pH decrease upon continued heating, which suggests that the blue pigment, or its neutral, yellow form, may account for some of the color products obtained in processing cottonseed.

26. BOATNER, C. H., O'CONNOR, R. T., CURET, M. C., and SAMUELS, C. S.

THE PIGMENTS OF COTTONSEED. III. GOSSYFULVIN, A NATIVE COTTONSEED PIGMENT RELATED TO GOSSYPOL. Amer. Chem. Soc. Jour. 69(6): 1268-1273. 1947.

Further insight into the structure of gossyfulvin and its relation to gossypol has been obtained. On the basis of its elementary composition and its yields of gossypol upon acid hydrolysis, the molecular formula, $\text{C}_{34}\text{H}_{34}\text{N}_2\text{O}_8$, has been proposed. Exact correspondence of the absorption spectra of gossyfulvin and dianilnogossypol, and their dissimilarity with that of gossypol, have been assumed as evidence that gossyfulvin and dianilnogossypol have identical basic structures and that both differ from the preponderant tautomeric form gossypol. Isomerism of the three oximes formed from gossypol, dianilnogossypol, and gossyfulvin seems evidence of

the liability of the carbonyl groups of gossypol. It indicated that the acetyl derivative formed in preponderant amounts upon acetylation of gossypol is tetraacetoxygossypol, and that formed from gossyfulvin is a hexacetyl derivative.

27. EAGLE, E., CASTILLON, L. E., HALL, C. M., and BOATNER, C. H.

ACUTE ORAL TOXICITY OF GOSSYPOL AND COTTONSEED PIGMENT GLANDS FOR RATS, MICE, RABBITS, AND GUINEA PIGS. Arch. Biochem. 18(2): 271-277. 1948.

Results are consistent with those obtained with chickens. Glands added to diets of fasting rats, mice, guinea pigs, and rabbits proved highly toxic, causing death with large doses, whereas an equivalent amount of pure gossypol affected the animals very little. Toxicity of pigment glands did not appear related to their contents of gossypol or gossypurpurin. These results add to the evidence that some component of the glands, other than, or in addition to gossypol and gossypurpurin is responsible for their toxicity. The toxic material is relatively stable to heat; but it is partly inactivated by the combined action of heat and water. It is not extracted from the glands by petroleum naphthas and tetrachlorethylene but is partly extracted by ethanol and is completely extracted by diethyl ether and acetone.

28. BOATNER, C. H., HALL, C. M., and MERRIFIELD, A. L. (Assignors to the United States of America as represented by the Secretary of Agriculture)

HEAVY GRAVITY LIQUID SEPARATION OF COTTON SEED. U. S. Patent 2,482,141. September 20, 1949.

This patent describes a process for separating the pigments from a cottonseed product containing substantially intact pigment glands embedded in the seed embryo tissues. The process comprises treating the product to disintegrate the embryo tissue and release the intact pigment glands, mixing with a liquid which does not substantially rupture the pigment glands and which has a density greater than that of the pigment glands to be separated and less than that of the remaining parts; and then settling the mixture so that the pigment glands float to the top where they can be removed.

29. CASTILLON, L. E., HALL, C. M., O'CONNOR, R. T., and MILLER, C. B.

EFFECT OF TEMPERATURE ON THE CONTENT OF PIGMENTS OF STORED COTTONSEED. Amer. Oil Chem. Soc. Jour. 26(11): 655-659. 1949.

Three pure-bred varieties of cottonseed, *G. hirsutum*, planted and grown under similar environmental conditions, were stored at 38°, 77°, and 85°F., after determination of their initial contents of lipids, nitrogen, moisture, gossypol, and gossypurpurin. The content of gossypurpurin in all the samples increased in proportion to the temperature and length of storage, while the content of gossypol decreased. During storage another yellow-colored pigment developed which could be separated from gossypol by alkaline extraction of the original chloroform extract of the stored seed. The alkali-extractable portion of the chloroform extract gave a red-colored antimony trichloride reaction product characteristic of gossypol. At least a fraction of the nonacidic pigment in the crude chloroform extracts obtained from stored cottonseed is thought to be diaminogossypol.

30. EAGLE, E.

DETOXIFICATION OF COTTONSEED PIGMENT GLANDS WITH FERROUS SULFATE. Soc. Expt. Biol. and Med. Proc. 2(2): 444-446. 1949.

Acute toxicity studies were made on 3 different samples of cottonseed pigment glands administered orally to 228 rats in the form of slurries made with either distilled water or 2 percent ferrous sulfate solution. The LD₅₀ values for the samples when administered in water were 1140, 1490, and 2290 mg./kg., respectively. When given in 2 percent ferrous sulfate solution, the samples were detoxified so that even such high doses as 7,000 mg./kg. for each were no longer toxic. There was little effect on the body weight of all 99 rats which survived doses of ferrous sulfate-treated cottonseed pigment glands 3 to 6 times the LD₅₀ value for water-suspended glands. Administration of pigment glands in 0.5 percent ferrous sulfate did not detoxify them and led to the death of all 24 rats given this preparation.

31. EAGLE, E., HALL, C. M., CASTILLON, L. E., and MILLER, C. B.

EFFECT OF FRACTIONATION AND TREATMENT ON THE ACUTE ORAL TOXICITY AND ON THE GOSSYPOL AND GOSSYPURPURIN CONTENT OF COTTONSEED PIGMENT GLANDS. Amer. Oil Chem. Soc. Jour. 27(8): 300-303. 1950.

Fractionated and variously treated cottonseed pigment gland preparations have now been tested, and the acute

oral toxicity of 6 different samples of pure gossypol has been determined. Toxicity of glands was not related to their extractable gossypol or gossypurpurin contents. Fractions of glands tested for toxicity showed that a water- and acetone-soluble fraction was more toxic than the original glands; but an acetone-insoluble residue had practically no toxicity. Dry heating had no apparent effect on toxicity of glands, whereas heating in the presence of water markedly decreased toxicity. Toxicity of pigment glands decreased with the time of storage of the original seed. The acute oral toxicity of pure gossypol in rats was less than that for preparations of glands which had extractable gossypol contents ranging from as little as 24 to 90 percent. Results again confirmed that toxicity of glands is attributable either to some component or components other than, or in addition to, gossypol, or to one which enhances the activity of gossypol.

32. POMINSKI, C. H., CASTILLON, L. E., and DECHARY, J. M.
REMOVING THE GLANDS FROM COTTONSEED.
U. S. Dept. Agr. Yearbook of Agr., 1950-51: 558-560.
1951.

Cottonseed pigment glands under the microscope appear brilliantly and variously colored, from yellow through orange, and red to purple. Small glands are almost spherical; large ones, more elongated. They are so small that all the glands in a single seed make up only 1 to 3 percent of its total weight; 40 to 59 percent by weight of the gland is wall, 35 to 50 percent is gossypol, and 0.05 to 3 percent is gossypurpurin. Colors are caused by variations in the relative amounts of each of the two pigments inside the gland. Walls of the glands resist the action of certain solvents and also are lighter in weight than the rest of the seed. It is possible to separate the tiny glands by causing them to float. This is called the gland-flotation process. The meal and oil are superior in many ways to those produced by older methods. The third product, the pigment glands, is entirely new. They are the only parts of cottonseed that as yet have no commercial value. The method developed to remove the glands has made available sufficient quantities of pigment glands, as well as of gland-free meal, for extensive investigations of their physical, chemical, and physiological properties.

33. AMBROSE, A. M., and ROBBINS, D. J.
STUDIES ON THE CHRONIC ORAL TOXICITY OF
COTTONSEED MEAL AND COTTONSEED PIGMENT
GLANDS. Jour. Nutrition 43(3): 357-370. 1951.

Results of feeding studies conducted on rats indicated the following:

Cottonseed meal free of pigment glands is devoid of any toxic principle as judged by the growth of rats and histopathological examination of visceral organs. Hexane-extracted meal is not completely devoid of toxic principles or appetite-depressing factors, as judged by inhibition in growth of rats on diets containing 15 percent or more of the meal.

Paired feeding studies with cottonseed pigment glands and gossypol indicate that the decrease in growth is not due entirely to reduced food intake, but that gossypol plays an important role in depressing growth. Toxicity of pigment glands and gossypol is low.

34. POMINSKI, C. H., and VON DER HAAR, P.
REACTIONS OF SOME GOSSYPOL-LIKE PIGMENTS
WITH ANILINE AND P-ANISIDINE. Amer. Oil Chem.
Soc. Jour. 28(10): 444-446. 1951.

Gossypurpurin and diaminogossypol reacted with aniline to give the same reaction product as does gossypol. They reacted with p-anisidine to give products whose absorption spectra exhibit maxima in the same positions as that of the reaction product of gossypol and p-anisidine.

35. POMINSKI, C. H., MILLER, C. B., VONDER HAAR, P.,
O'CONNOR, R. T., CASTILLON, L. E., and BROWN,
L. E.
PIGMENTS OF COTTONSEED. IV. GOSSYPUR-
PURIN, A PURPLE PIGMENT RELATED TO GOSSY-
POL. Amer. Oil Chem. Soc. Jour. 28(11): 472-475.
1951.

Properties of gossypurpurin prepared from gossypol via diaminogossypol were compared with those of gossypurpurin isolated from pigment glands. A tentative molecular formula for synthetic gossypurpurin has been proposed on the basis of its elementary composition. The native pigment could not be obtained in the same degree of purity as the synthetic product and analytical data could not be brought into exact agreement. However, solutions of both pigments in chloroform

gave almost identical absorption spectra and antimony trichloride tests. Qualitative reactions indicated that the functional groups of both the native and the synthetic gossypurpurin are identical, and the ready conversion of both products to gossypol upon contact with acid indicated that their basic structures are similar.

36. VON DER HAAR, P., and POMINSKI, C. H.
PIGMENTS OF COTTONSEED. V. METHYLATION
OF GOSSYPURPURIN. Jour. Organic Chem. 17(2):
177-180. 1952.

The methylation of gossypol gave the completely methylated ether, hexamethoxygossypol. Methylation of gossypurpurin gave a product whose methoxyl value indicated the presence of 4 methoxyl groups and which resisted further methylation. The molecular size of the gossypurpurin methyl ether was approximately twice that of hexamethoxygossypol. A tentative molecular formula for the gossypurpurin methyl ether was proposed on the basis of its elementary analysis, methoxyl value, and molecular weight.

SEE ALSO: 13, 38, 39, 40, 46, 47, 48, 49, 50, 56, 72,
73, 75, 80, 104, 115, 117, 134, 139.

III. GOSSYPOL

37. BOATNER, C. H., CARAVELLA, M., and KYAME, L.
QUANTITATIVE DETERMINATION OF EXTRACT-
ABLE GOSSYPOL IN COTTONSEED AND COTTON-
SEED MEAL. A SPECTROPHOTOMETRIC METHOD.
Indus. and Engin. Chem., Analyt. Ed. 16(9): 566-572.
1944.

An accurate spectrophotometric method for determination of extractable gossypol in nondefatted cottonseed meal is described. The reaction of gossypol with antimony trichloride in a hydrochloric acid-treated chloroform or ether extract of cottonseed meal forms a soluble red product. This exhibits a characteristic absorption curve with a maximum at 510-520 $m\mu$. The gossypol concentration is proportional to the extinction at the absorption maximum. Accurate and reproducible results can be obtained.

38. CASTILLON, L. E., HALL, C. M., and BOATNER, C. H.
PREPARATION OF GOSSYPOL FROM COTTONSEED
PIGMENT GLANDS. Amer. Oil Chem. Soc. Jour.
25(7): 233-236. 1948.

Because the nature of gossypol, as yet difficult to obtain in pure form, suggests many possibilities for use as an antioxidant and possibly in the pharmaceutical field, practical methods were sought for its extraction from cottonseed pigment glands and for its isolation from pigment gland extracts. The pigment glands used in the experiments were produced from whole cottonseed meal and from defatted cottonseed meal by the gland flotation process. Of the methods developed during the investigation, extraction with acetone followed by precipitation of gossypol acetic acid from the extract was found to be the preferable one for obtaining pure gossypol in good yields. Since this method is rapid and employs inexpensive and easily handled reagents, it is readily adaptable to the production of gossypol on a large scale. Precautions that must be taken in applying the method to large-scale production are discussed together with methods for the purification and preservation of gossypol.

39. HALL, C. M., CASTILLON, L. E., GUICE, W. A., and
BOATNER, C. H.
APPLICATION OF THE ANTIMONY TRICHLORIDE-
SPECTROPHOTOMETRIC METHOD TO THE DE-
TERMINATION OF GOSSYPOL IN COTTONSEED AND
COTTONSEED PRODUCTS. Amer. Oil Chem. Soc.
Jour. 25(12): 457-461. 1948.

Method described for application of the antimony trichloride spectrophotometric method for the

determination of gossypol in a variety of cottonseed products. The determination involved the following operations: extraction of sample with chloroform or aqueous ethanol; isolation of gossypol from the extracts by use of aqueous alkali containing sodium dithionite; separation of the gossypol from the aqueous alkali by acidification and subsequent recovery in chloroform; application of the antimony trichloride spectrophotometric method to the latter chloroform solution. This modified method was more precise and accurate for determination of gossypol in cottonseed products than original method. Data were given to show the results obtained upon testing of isolated pigment glands, seed, various types of cottonseed meals, and oils with this modified procedure.

40. BOATNER, C. H., CASTILLON, L. E., HALL, C. M., and NEELY, J. W.

GOSSYPOL AND GOSSYPURPURIN IN COTTONSEED OF DIFFERENT VARIETIES OF G. BARBADENSE AND G. HIRSUTUM, AND VARIATION OF THE PIGMENTS DURING STORAGE OF THE SEED. Amer. Oil Chem. Soc. Jour. 26(1): 19-25. 1949.

A number of varieties of upland cottonseed (G. hirsutum), having different varietal characteristics with respect to length of fiber and contents of lipids and nitrogen, as well as several varieties of Egyptian and Sea Island seed were planted and grown under as nearly identical conditions as possible for a systematic investigation of the influence of genetic factors on the pigment content of the kernels. The initial contents of moisture, lipids, nitrogen, gossypol, and gossypurpurin were determined, after which samples of all of the seed were stored for a year at 80°F. and analyzed periodically for gossypol and gossypurpurin. A definite relation was found between the species of the seed and their gossypol and gossypurpurin contents. Sea Island and Egyptian seed of the species G. barbadense contained more gossypol and very much more gossypurpurin than seed of the species G. hirsutum. Within the species G. barbadense Sea Island seed contained more gossypol and less gossypurpurin than Egyptian seed. Gossypurpurin increased during storage of all of the seed whereas gossypol varied in a number of ways, increasing in some, decreasing in others, and remaining relatively constant in a few samples of stored seed. Gossypol was determined on the basis of the specific extinction coefficients at 366 m μ in chloroform extracts of the seed, and also by means of the antimony trichloride method. The relatively large and inconsistent changes in pigmentation

of seed from late planting demonstrated that gossypol and gossypurpurin were independently affected by conditions of growth and maturity. Some evidence was presented that the intraglandular pigments participate in the metabolism of the seed. It was suggested that gossypurpurin, or a decomposition product of this pigment, was responsible for the dark colors of oils produced from Sea Island and Egyptian seed, as well as those produced from stored cottonseed.

41. PONS, W. A., JR., and GUTHRIE, J. D.
DETERMINATION OF FREE GOSSYPOL IN COTTON-
SEED MATERIALS. Amer. Oil Chem. Soc. Jour.
26(11): 671-676. 1949.

A method for the determination of free gossypol in cottonseed materials is described. The gossypol is extracted with a measured volume of 70 percent aqueous acetone on a shaker for one hour, then is filtered, and a colorimetric analysis of the filtrate made by means of the reaction between gossypol and *p*-anisidine. The conditions for complete extraction of gossypol from various types of cottonseed materials were investigated, and the stability of gossypol in aqueous acetone was demonstrated. Data also are presented on the recovery of gossypol added to cottonseed materials.

42. EAGLE, E.
CHRONIC TOXICITY OF GOSSYPOL. Science 109
(2832): 361. 1949.

Because of the widespread publicity attained by gossypol as an appetite depressant and the relatively large acute dosages of pure gossypol needed to produce toxic symptoms in test animals, the chronic toxicity of pure gossypol for dogs was determined. Four young, litter-mate male dogs were given daily doses of 0, 50, 100 and 200 mg. pure gossypol per kg. body weight of the dogs during three different experimental periods: 55-day control period, 5-day experimental period (gossypol by capsule), 9-day control (rest) period, 5-day experimental period (gossypol by stomach tube), 9-day control period, 9-day experimental period (gossypol by stomach tube). Each dog had received a total of 19 daily doses of gossypol within a period of 37 days, which resulted in death of all three dogs within 5 days after the last dose (one on the fourth and two on the fifth day). Dogs fed the repeated doses of gossypol exhibited the toxic symptoms attributed to pigment gland poisoning and showed marked weight losses. Post-mortem examination

showed the same pathological findings associated with cottonseed or gossypol poisoning for rats, mice, guinea pigs, and rabbits. It was suggested that use of gossypol with human subjects be withheld.

43. EAGLE, E., and BIALEK, H. F.

EFFECT OF SINGLE AND REPEATED DOSES OF GOSSYPOL ON THE RAT. Food Res. 15(3): 232-236. 1950.

The effects of single doses of pure gossypol on the body weights of fasting and nonfasting rats were determined; the effects of 11 consecutive small dosings of gossypol on body weights of nonfasting rats were also determined. LD₅₀ values were calculated according to the method Reed and Muench. In the nonfasting rat single doses of 50, 100 and 200 mg. gossypol (LD₅₀ 2,800 mg./kg.) per kg. body weight and 100 mg. pigment glands (LD₅₀ 1,140 mg./kg.) per kg. body weight containing 40 percent gossypol showed no effects on body weights. Eleven consecutive daily doses of same level in the same rats caused body weight depressions proportional to amount of gossypol administered. As little as 50 mg./kg. of pure gossypol (1/56 of its oral LD₅₀ value) given daily for 11 days caused body weight depression below that of controls. In fasting rats, single doses of pure gossypol at 15 different dosage levels from 100 to 2,400 mg./kg. caused 25 percent weight losses within 6 days. Starvation controls lost more than 31 percent weight during the same period. Single doses of 500 mg. gossypol/kg. body weight or less did not prevent good recovery to prefasting body weight on or before 6th day of dosing. Rats given single doses of 600 mg. gossypol/kg. required a longer period of recovery time.

44. EAGLE, E.

EFFECT OF REPEATED DOSES OF GOSSYPOL ON THE DOG. Arch. Biochem. 26(1): 68-71. 1950.

Repeated daily doses of gossypol by stomach tube at levels of 10, 15, 30, 50, 100, and 200 mg./kg. for periods varying from 5 to 12 days caused marked diarrhea, anorexia, and weight loss, and finally led to the death of 6 dogs. Repeated doses of 1, 2, and 5 mg./kg./day for similar periods produced only slight decreases in food intake and body weight and did not prove fatal.

45. CASTILLON, L. E., and ALTSCHUL, A. M.
PREPARATION OF WATER-SOLUBLE COMBINATION PRODUCTS OF GOSSYPOL AND THEIR TOXICITY TO AQUARIUM FISH. Soc. Expt. Biol. and Med. Proc. 74(3): 623-626. 1950.

The purpose of this investigation was to make combination products of gossypol with naturally occurring materials which would have physical properties different from those of the original material and to determine the effect of such changes on some physiological actions of gossypol. Gossypol was tested on aquarium goldfish and sharp differences in physiological activity between the original gossypol and the combination products were obtained. Gossypol was combined with proteins, amino acids, and carbohydrates, to produce materials that were soluble or dispersible in water or buffer solutions at pH 7.0 by mixing gossypol and the other material in aqueous solution at alkaline pH, neutralizing that mixture, and drying by lyophilization. These water-soluble products were lethal to goldfish at concentrations as small as 1 part in 100,000 parts of water. An aqueous solution of gossypol-peanut protein maintained its toxicity and its original gossypol content for at least 48 hours. Cottonseed pigment glands were shown to be less toxic than the gossypol combination complexes. Crystalline gossypol, when added to the aquarium bath, displayed no visible toxic effects on the goldfish.

46. LILLIE, R. J., and BIRD, H. R.
EFFECT OF ORAL ADMINISTRATION OF PURE GOSSYPOL AND OF PIGMENT GLANDS OF COTTONSEED ON MORTALITY AND GROWTH OF CHICKS. Poultry Sci. 29(3): 390-393. 1950.

The toxicity of cottonseed pigment glands administered by capsule was due entirely to the gossypol content (33 percent). Gossypol administered by capsule inhibited growth of New Hampshire chicks at a level of 0.063 percent of the diet.

47. PONS, W. A., JR., HOFFPAUIR, C. L., and O'CONNOR, R. T.
DETERMINATION OF TOTAL GOSSYPOL PIGMENTS IN COTTONSEED MATERIALS. Amer. Oil Chem. Soc. Jour. 27(10): 390-393. 1950.

A method for the determination of total gossypol pigments in cottonseed materials is described which involves an acid hydrolysis of the "bound" gossypol

pigments in aqueous methyl ethyl ketone, and the color-metric determination of the gossypol pigments by the method described by Pons and Guthrie (See Item 41) for free-gossypol pigments. The conditions for hydrolysis have been investigated for various types of cottonseed materials. Data are presented on the recovery of gossypol added to such materials. Spectrophotometric data indicated that the pigments in the hydrolyzates are gossypol and closely related pigments and that the conditions specified not to alter them.

48. PONS, W. A., JR., HOFFPAUIR, C. L., and O'CONNOR, R. T.

DETERMINATION OF TOTAL GOSSYPOL PIGMENTS IN COTTONSEED OILS. Amer. Oil Chem. Soc. Jour. 28(1): 8-12. 1951.

A method is proposed for the application of the p-anisidine-gossypol reaction to the analysis of cottonseed oils for total gossypol pigments. The oil is dissolved in a hexane-isopropanol solvent and gossypol pigments are determined in an aliquot of the solution by means of the color developed with p-anisidine. The effect of the solvent and of the oil concentration on the color developed in the method has been investigated. The stability of gossypol in the solvent employed has been demonstrated. Data are presented on the recovery of gossypol added to cottonseed oils. Further spectrophotometric evidence indicated that a number of gossypol-like pigments are present in the crude oils and that they react with p-anisidine to give reaction products spectrophotometrically identical with that obtained for pure gossypol with the same reagent.

49. HOPPER, T. H.

NEW METHODS DEVELOPED FOR DETERMINING GOSSYPOL IN COTTONSEED. U. S. D. A., Agr. Res. Admin., Res. Achvt. Sheet 145(C), 2 pp. July 1951.

Three new speedy analytical methods described that give accurate, reproducible determinations of gossypol pigments in cottonseed and cottonseed products were developed at the Southern Regional Research Laboratory. Two methods give information on the total amount of gossypol and gossypol-like substances present in cottonseed products; the third method shows the amount of free gossypol.

50. HOPPER, T. H.

REVIEW OF METHODS OF ANALYSIS OF COTTON-SEED MEALS. Oil Mill Gazetteer 55(9): 15-16. 1951.

Discussion of consideration of some aspects of the laboratory estimation of protein quality and of the determination of gossypol pigments in connection with the work of the Southern Laboratory on increasing the utility and nutritive value of cottonseed meal is given.

51. ALTSCHUL, A. M., and CASTILLON, L. E. (Assignors to the United States of America as represented by the Secretary of Agriculture)

WATER-DISPERSIBLE COMBINATION PRODUCTS OF GOSSYPOL AND CARBOHYDRATES. U. S. Patent 2,563,808. August 14, 1951.

Mixing gossypol and carbohydrates (potato starch, dextrose) in basic aqueous solution, neutralizing, and drying without heat, produces water-dispersible combination products having physiological properties different from gossypol.

52. POMINSKI, C. H., CASTILLON, L. E., VON DER HAAR, P., BROWN, L. E., and DAMARE, H.

STORAGE OF GOSSYPOL. Amer. Oil Chem. Soc. Jour. 28(8): 352-353. 1951.

Pure gossypol was stored for 18 months at temperatures of 3° and 23°-28°C. in the presence or absence of light and air. On the basis of combustion data, absorption spectra, antimony trichloride tests, and melting points of the original and stored samples it can be concluded that gossypol can be stored without deterioration for at least 18 months at temperatures of from 3° to 23°-28°C. in the absence of light, irrespective of the presence or absence of air.

53. HARMS, W. S., and HOLLEY, K. T.

HYPOPROTHROMBINEMIA INDUCED BY GOSSYPOL. Soc. Expt. Biol. and Med. Proc. 77(2): 297-299. 1951.

Gossypol induces hypoprothrombinemia in rabbits and pigs. Cottonseed meal containing free gossypol induces hypoprothrombinemia in young rabbits which begins after about 7 days' feeding and passes through a maximum at approximately 17 days then slowly returns to normal around 28 days. Gossypol is not as effective an anticoagulant as Dicumarol but acts somewhat more rapidly.

54. ALTSCHUL, A. M., and CASTILLON, L. E. (Assignors to the United States of America as represented by the Secretary of Agriculture)
WATER-SOLUBLE COMBINATION PRODUCTS OF GOSSYPOL AND PROTEINS. U. S. Patent 2,581,342. January 8, 1952.

The products are toxic to goldfish and are prepared by dissolving gossypol and proteins, such as peanut or casein proteins, in a basic aqueous medium, neutralizing, and freezing the medium, and removing the water by sublimation.

55. DECHARY, J. M., KUPPERMAN, R. P., THURBER, F. H., and ALTSCHUL, A. M.
REMOVAL OF GOSSYPOL FROM COTTONSEED BY SOLVENT EXTRACTION PROCEDURES. Amer. Oil Chem. Soc. Jour. 29(8): 339-341. 1952.

The relative efficiencies of organic, polar solvents, and of solvent-water pairs for use in the extraction of gossypol and related compounds from cottonseed flakes were determined in a specially devised glass laboratory extractor. A butanone-water pair containing 10 percent of water by volume was the most effective, and chlorine-substituted hydrocarbons were the least effective. Flakes extracted at 26°C. contained 0.08 percent free gossypol and those extracted at 71°C., 0.054 percent. This decrease may be due, in part, to the reaction of gossypol with the protein to form bound gossypol.

56. EAGLE, E., and BIALEK, H. F.
TOXICITY AND BODY WEIGHT-DEPRESSING EFFECTS IN THE RAT OF WATER-SOLUBLE COMBINATION PRODUCTS OF GOSSYPOL, GOSSYPOL, AND COTTONSEED PIGMENT GLANDS. Food Res. 17(6): 543-549. 1952.

The acute oral toxicities to rats and effects on their body weights of pure gossypol and combination products of gossypol with glycine, dextrose, and peanut protein, and the subacute toxicities of pure gossypol and pigment glands were reported. In acute toxicity studies, single doses of test materials (10 percent aqueous solutions or suspensions) were given by stomach tube. Daily weights were recorded for at least 2 weeks after dosing. LD₅₀ values were calculated according to method of Reed and Muench. The stored gossypol-peanut protein killed test animals whereas gossypol-glycine or gossypol-dextrose (stored) containing equivalent amount of

extractable gossypol did not kill animals. The acute oral toxicities of the combination products were not proportional to their gossypol contents. The combination products were less toxic to rats than pigment glands containing a smaller extractable gossypol content. The depressions in body weight caused by single doses of any of the combination products were proportional to the gossypol equivalent administered; but, as a group, the different gossypol combination products varied in their effect on body weight in a manner not related to the gossypol equivalents administered. Pigment glands showed poor correlation between their acute oral toxicities and their extractable gossypol contents. Subacute toxicity test on rats fed different levels of gossypol ranging from 0.05 to 1.34 g. per 100 g. stock diet per day, or 1.34 g. pigment glands containing 21.85 percent gossypol and 0.6 and 0.9 g. glands containing 32.22 percent gossypol showed the following results: The weight depression was attributable to the amount of gossypol contained in daily diets; however, the subacute toxicities of pigment glands could not be attributed to their gossypol content alone. It was suggested that some other factor in glands was responsible for their toxicity.

57. KING, W. H., and THURBER, F. H.

AN IMPROVED PROCEDURE FOR THE PURIFICATION OF GOSSYPOL. Amer. Oil Chem. Soc. Jour. 30(2): 70-74. 1953.

Methods of dissociating the gossypol-acetic acid complex prepared from the butanone extract of defatted cottonseed flakes and methods of recrystallizing gossypol were studied. The study led to the development of an improved rapid procedure for the purification of gossypol. In this procedure gossypol-acetic acid is dissociated with aqueous sodium hydroxide containing sodium hydrosulfite; the gossypol is precipitated with hydrochloric acid, extracted with ether, and recrystallized from a mixture of ether and xylene. Two crystallizations by this procedure gave gossypol free from acetic acid and of a high degree of purity. Gossypol-acetic acid of a high degree of purity was prepared by a modification of this procedure. The degree of purity of the gossypol prepared by the improved procedure was determined by a number of tests, which are described. Spectral and titration curves for pure gossypol are presented, and standards of purity are suggested. The molecular weight of gossypol and gossypol-acetic acid prepared by the procedures described is approximately 518 and 578, respectively.

58. PONS, W. A., JR., HOFFPAUIR, C. L., and HOPPER, T. H.

GOSSYPOL IN COTTONSEED: INFLUENCE OF VARIETY OF COTTONSEED AND ENVIRONMENT. Jour. Agr. and Food Chem. 1(18): 1115-1118. 1953.

The first systematic investigation of the influence of variety of cottonseed and environment on the gossypol content of kernels is reported. Data are reported for the gossypol content of kernels from the seed of 8 varieties of cotton grown at 13 locations during 3 years. Values ranged from 0.39 to 1.70 percent gossypol in the moisture-free kernels. Analysis of variance showed that both variety and environment significantly influenced those values. Standard deviations of the means suggested that these effects are about equal in their influence. (This paper is one of a series reporting the results of a Regional Cottonseed Study, 1947-1949. H. D. Barker and his associates of the Crops Research Division of the Agricultural Research Service have cooperated.) (See also item 60.)

59. HOFFPAUIR, C. L., and PONS, W. A., JR.

REVIEW OF THE PROPERTIES OF GOSSYPOL AND METHODS OF ITS ESTIMATION. Assoc. Off. Agr. Chem. Jour. 36(4): 1108-1118. 1953.

This article reviews 47 references giving information on the physical and chemical properties of gossypol and analytical methods for its determination. The article is illustrated by 4 figures, showing (1) tautomeric forms of gossypol; (2) ultraviolet absorption spectra of gossypol in cyclohexane; (3) ultraviolet absorption spectra of cottonseed pigments in chloroform; and (4) absorption spectra of 70 percent aqueous acetone extracts of various cottonseed materials reached with p-anisidine.

60. HOFFPAUIR, C. L.

INFLUENCE OF VARIETY AND ENVIRONMENT ON THE GOSSYPOL CONTENT OF COTTONSEED KERNELS. Coop. Cottonseed Oil Mills Conf. Jour. of Proc. 5: 13-14. New Orleans, La., 1953.

Samples of seed from 8 commercial varieties of cotton grown at 13 locations during 1947, 1948, and 1949 were obtained and analyses were conducted on kernels which were separated from the seed.

Wide variations in gossypol content ranging from 0.39 to 1.70 percent on a moisture-free basis were observed.

Analysis of variance indicated that both variety and environment have a highly significant influence on the gossypol content of the kernel, with environment having a slightly greater effect on the gossypol content than the varietal characteristics.

Gossypol in the kernel was found to be negatively correlated with temperature and positively correlated with rainfall, higher correlation coefficients being found for the maturation period during which gossypol is synthesized in the seed than for the earlier periods of seed development.

Correlations between the gossypol content of individual varieties with light temperature and rainfall shows differences in varietal response to these environmental factors. Varieties low in gossypol content were affected to a greater extent by temperature than were the varieties high in gossypol content. All varieties were significantly affected by rainfall. Similar relations were observed when the gossypol values were calculated to an oil-free basis. (See also Item 58.)

61. CASTILLON, L. E., KARON, M., ALTSCHUL, A. M.,
and MARTIN, F. N.
PREPARATION OF COMBINATION PRODUCTS OF
GOSSYPOL WITH GLYCINE AND DEXTROSE AND
THEIR TOXICITY TOWARD MICE. Arch. Biochem.
and Biophys. 44(1): 181-188. 1953.

Combination products of gossypol with glycine and with dextrose have been prepared. Acute oral toxicity investigations indicate that the freshly prepared gossypol-dextrose combination product is more toxic than isolated gossypol and as toxic as isolated pigment glands. The gossypol-glycine combination product is nontoxic when administered in dosages as large as 12.0 g./kg. body weight of mice. Purified gossypol-glycine has been isolated and found to be a water-soluble, nitrogen-containing compound. It differs from gossypol in many of its properties.

62. BICKFORD, W. G., PACK, F. C., CASTILLON, L. E.,
and MACK, C. H.
THE ANTIOXIDANT AND ANTIPOLYMERIZATION
PROPERTIES OF GOSSYPOL, DIANILINO GOSSYPOL,
AND RELATED MATERIALS. Amer. Oil Chem. Soc.
Jour. 31(3): 91-93. 1954.

In an attempt to find a commercial use for gossypol, the antioxidant and antipolymerization properties of

gossypol, dianilinogossypol, gossypol-urea and gossypol-glycine combination products, and gossypolaminobenzenethiel were investigated. Antioxidant properties of 0.05 percent ethanol solutions of test samples were evaluated in: (1) refined, bleached, deodorized cottonseed oil having an initial peroxide value of 0, and in (2) commercial prime, steam-rendered lard, free of added antioxidants and peroxides. Hydroquinone was included in both tests as control sample for comparison purposes. Peroxide values were determined according to Moore and Bickford method. The effectiveness of the test materials as inhibitors of polymerization in the monomers--styrene, vinyl acetate, and methyl acrylate--was measured as changes in viscosity with Hardner-Holdt bubble standard tubes. Polymerization was produced by ultraviolet light at room temperature and by heat treatment in a thermostatically controlled water bath. The effectiveness of the various gossypol-reaction products as antioxidants was roughly equivalent to gossypol on a molar basis. Dianilinogossypol at 0.05 percent level was a very effective polymerization inhibitor; at a 0.01 percent level it was as nearly effective as hydroquinone at 10 times that concentration. Gossypol was less effective. The gossypol products imparted orange or yellow colors to the monomers. Gossypol was used at concentrations below those reported to be physiologically active.

63. O'CONNOR, R. T., VON DER HAAR, P., DUPRÉ, E. F., BROWN, L. E., and POMINSKI, C. H.
THE INFRARED SPECTRA OF GOSSYPOL. Amer. Chem. Soc. Jour. 76(9): 2368-2373. 1954.

Infrared spectra of chloroform solutions of gossypol, 13 derivatives and degradation products of gossypol, and 18 "model" compounds containing groupings present in the gossypol molecule, have been obtained in the region from 2 to 12 $m\mu$. From wave length positions of observed maxima, correlations have been made with vibrating groups giving rise to the most prominent bands in the spectra. The infrared spectra confirm the fact that gossypol is an aromatic and phenolic compound. Correlation also confirms the presence of a carbonyl ortho to a hydroxyl group and in some derivatives of gossypol the presence of no carbonyl groups, and of carbonyl groups attached directly to an alicyclic grouping in others. The infrared spectra thus strongly support the chemical evidence that gossypol occurs in tautomeric forms. These observations are in agreement with the accepted structure of the gossypol

molecule described by R. Adams, et al. (p. 2368). However, no evidence was obtained from the infrared spectra regarding the binaphthyl character of gossypol. Hence, evidence for the binaphthyl group remains, as far as spectrophotometry is concerned, with ultraviolet spectra. No evidence was obtained from the infrared data for the presence of an isopropyl group. The correlations between the infrared spectra and chemical groupings in the molecules of gossypol and its derivatives provide a basis for following by infrared absorption the changes occurring in these and related compounds encountered in research on cottonseed and cottonseed products.

64. STORHERR, R. W., and HOLLEY, K. T.
FEED ANALYSIS: DETERMINATION OF FREE GOSSYPOL IN MIXED FEEDS. Jour. Agr. and Food Chem. 2(14): 745-747. 1954.

With less toxic cottonseed meals becoming commercially available, methods for free-gossypol content assay in mixed feeds are needed for experimental use and for control. The method here proposed is based on the color developed in the reaction of phloroglucinol with gossypol in strong acid at room temperature.

Interference by reactive substances brought in by the solvent is minimized by carrying out the reaction at room temperature. Results on meals are in substantial agreement with those obtained by the p-anisidine method (See Items 41 and 48). This method is not applicable to cottonseed meals treated with aniline, because the dianilinogossypol would be determined as free gossypol.

65. KUPPERMAN, R. P., and KARON, M. L.
EFFECT OF TIME AND TEMPERATURE OF STORAGE ON THE FREE AND TOTAL GOSSYPOL CONTENT OF COTTONSEED MEATS AND OF MIXED DIETS. Amer. Oil Chem. Soc. Jour. 32(2): 54-57. 1955.

With different types of cottonseed products and cottonseed products to which gossypol is added, there is a loss or disappearance of gossypol upon storage, as determined analytically. The rate of disappearance of gossypol varied for the different type cottonseed and was increased at higher temperatures and over the longer periods of storage.

When gossypol is incorporated into different types of animal diet materials, there is a loss or inactivation of some portion of the added gossypol as determined analytically immediately after preparation of the gossypol-diet mixtures, and a further additional loss or inactivation upon storage. The factors that may contribute to this loss or inactivation of gossypol are: a) the components of the diet materials; b) the temperature and length of time of storage; c) the concentration of gossypol added and the final percentage of gossypol in the mixture.

66. PONS, W. A., JR. and HOFFPAUIR, C. L.
DETERMINATION OF FREE GOSSYPOL IN CHEMICALLY TREATED COTTONSEED MEALS CONTAINING DIANILINO GOSSYPOL. Amer. Oil Chem. Soc. Jour. 32(5): 295-300. 1955.

A method is proposed for the determination of free gossypol in chemically treated cottonseed meals containing dianilinogossypol. The procedure includes a rapid qualitative test for detecting the presence of dianilinogossypol in cottonseed meals.

67. WORONICK, C. L., and GRAU, C. R.
COTTONSEED MEAL IN POULTRY FEED: GOSSYPOL-CEPHALIN COMPOUND FROM FRESH EGGS OF HENS FED COTTONSEED MEAL. Jour. Agr. and Food Chem. 3(8): 706-707. 1955.

When gossypol is present in egg yolk, it is present not in free form as postulated, but in bound form. It has been found that egg cephalin consists almost entirely of phosphatidylethanolamine. As gossypol readily reacts with amino groups, it is concluded that the primary amino groups of egg cephalin condense with the aldehyde groups of gossypol to form a Schiff base.

Unpublished results have shown that egg yolk protein contains bound gossypol. The gossypol, which is probably attached to the free amino groups of the protein, can be released and identified by oxalic hydrolysis followed by spectrophotometry.

The results indicate that amino groups play an important role in the metabolism of gossypol by the laying hen. Preliminary paper presented Poultry Science Association, July 1954; abstract in Poultry Sci. 33(5): 1088. 1954.

68. HOFFPAUIR, C. L., and PONS, W. A., JR.
REPORT ON GOSSYPOL IN FEEDS. EFFECT OF
VARIATION IN SAMPLE WEIGHTS AND ALIQUOTS
ON DETERMINATION OF FREE GOSSYPOL. Assoc.
Off. Agr. Chem. Jour. 38(2): 234-238. 1955.

The application of accepted methods for the determination of free gossypol in cottonseed meal to the analysis of mixed feeds requires the use of increased sample weights or increased aliquots or both. Modification of the color development to use p-anisidine at 80°C. permits the use of larger aliquots but the sample size is limited to 1 gm. or less as the use of larger samples results in incomplete extraction of the free gossypol.

69. JONASSEN, H. B., and DEMINT, R. J.
INTERACTION OF GOSSYPOL WITH THE FERROUS
ION. Amer. Oil Chem. Soc. Jour. 32(7): 424-426.
1955.

Several investigators have used iron salts to inactivate gossypol, and the object of this investigation was to determine the combining ratios for the reaction between gossypol and the ferrous ion. The preparation of a solution of sodium gossypolate in aqueous acetone has been described. Indications of a 1:1 mole ratio combination of the gossypolate and ferrous ions were established through conductometric and potentiometric studies in the presence of either chloride or perchlorate anion. Application of the method of continuous variations to differences in the absorption spectra of the two species confirmed the 1:1 mole ratio of ferrous ion to gossypol. This finding is supported by analytical data. Values of pK for the dissociation constant of ferrous gossypolate, calculated from spectrophotometric measurement, averaged 7.3.

70. SHIRLEY, D. A., and SHEEHAN, W. C.
REDUCTION OF GOSSYPOL WITH LITHIUM ALUMI-
NUM HYDRIDE. Amer. Chem. Soc. Jour. 77(17):
4606-4608. 1955.

The reduction of gossypol with lithium aluminum hydride followed by acetylation yields a product, designated as methylapogossypol hexaacetate, in which the aldehyde groups of gossypol have been reduced to methyl groups and all hydroxyl groups acetylated. Oxidation of methylapogossypol hexaacetate yields methylapogossypolone tetraacetate. Reductive acetylation of this latter product leads to methylapohydrogossypolone octaacetate.

71. SHIRLEY, D. A., and DEAN, W. L.

STRUCTURE AND REACTIONS OF GOSSYPOL.
II. THE SYNTHESIS OF 1, 6, 7- TRIMETHOXY-3-
METHYLNAPHTHALENE, A PROTOTYPE OF
DESAPOGOSSYPOL HEXAMETHYL ETHER. Amer.
Chem. Soc. Jour. 77(22): 6077-6079. 1955.

Continued investigations into the structure of gossypol are reported. An attempt was made to synthesize a compound more closely related to gossypol than desapogossypolone hexamethyl ether, one that would provide additional information on what is generally assumed to be the molecular structure of gossypol. 3-methyl-6, 7-dimethoxy-1-tetralone was converted, through several reactions, to obtain 1,6,7-trimethoxy-3-methylnaphthalene. At this point it was found that the ultraviolet absorption spectrum of this differed appreciably from that of desapogossypol hexamethyl ether. This difference in the absorption spectra was interpreted as indicating an attachment of the two naphthol rings through the 3,3'-positions rather than through the 2,2'-positions.

SEE ALSO: 14, 15, 20, 25, 27, 29, 31, 36, 72, 76, 80,
82, 86, 89, 91, 92, 93, 96, 97, 102, 104,
110, 118, 122, 125, 128, 129, 134, 135,
136, 137, 139, 140, 141, 145.

IV. PROCESSING

Effect on meal

72. BOATNER, C. H., HALL, C. M., O'CONNOR, R. T., CASTILLON, L. E., and CURET, M. C.
PROCESSING OF COTTONSEED. I. PIGMENT DISTRIBUTION IN OILS AND MEALS PRODUCED BY HYDRAULIC AND SCREW PRESS METHODS. Amer. Oil Chem. Soc. Jour. 24(4): 97-106. 1947.

Changes that occur in gossypol and other pigments during cooking and pressing of cottonseed by hydraulic and continuous screw-press methods were investigated. Laboratory-scale experiments were conducted on the effect of cooking, and mill-scale experiments on the effect of cooking and pressing. The pigmentation of cooked cottonseed was shown to depend upon moisture content and period of heating. Screw-pressed crude oils were more deeply colored and contained one principal pigment, whereas hydraulic-pressed oils contained two principal pigments. The absence of significant amounts of gossypol in the crude oils was demonstrated by means of a new technique for the quantitative isolation of gossypol. Crude-oil pigments differed from gossypol, but like gossypol, were removed during alkali refining. The pigmentation of crude oils has been shown to depend principally upon that of the original seed and the moisture content of the seed during cooking. Absorption spectra indicated that alkali-refined, hydraulic-pressed oils contain two or three pigments originally present in crude oils, whereas alkali-refined, screw-pressed oils contain, in addition, a large number of decomposition products of the principal crude-oil pigment.

73. BOATNER, C. H., HALL, C. M., O'CONNOR, R. T., CASTILLON, L. E., and CURET, M. C.
PROCESSING OF COTTONSEED. II. FACTORS DETERMINING THE DISTRIBUTION AND PROPERTIES OF PIGMENTS IN PRODUCTS PREPARED BY SOLVENT EXTRACTION. Amer. Oil Chem. Soc. Jour. 24(8): 276-283. 1947.

On the basis of the properties of the pigments and pigment glands of cottonseed, three methods were proposed for control of color in cottonseed products. Method (1): For extraction of oil essentially free of pigments,

moisture was excluded from the system and solvents other than water-miscible alcohols and ketones were used. The pigments remained in the meal. Their distribution in the meal, intact glands or absorption onto extraglandular tissue was dependent upon the amount of moisture in the system, and the temperature and duration of extraction. Supplementary extraction of the defatted cottonseed meal with methanol, ethanol, isopropanol, acetone or 1,4-dioxane removed gossypol from the meal; acetone and 1,4-dioxane were effective for removal of gossypurpurin from such meals. Method (2): Extraction of oil containing all of the seed pigments with water-miscible solvent to cause rupture of the pigment glands. Pigments are readily removed from these oils by usual refining procedures. The amount of pigmentation in the solvent-extracted meals was found to be dependent upon original seed pigmentation, and solubility of gossypol and gossypurpurin in solvents employed. Method (3): The gland-flotation process yielded intact pigment glands, and oil and meal essentially pigment-free. The development and fixation of color in solvent-extracted oils was not believed due to gossypol, but due to an oil-soluble, extraglandular pigment.

74. WILLIAMS, P. A., BOATNER, C. H., HALL, C. M., O'CONNOR, R. T., and CASTILLON, L. E.
PROCESSING OF COTTONSEED. III. COLOR DEVELOPMENT IN COTTONSEED OIL DURING STORAGE OF THE SEED AND CRUDE OIL. Amer. Oil Chem. Soc. Jour. 24(11): 362-369. 1947.

Hydraulic pressing of cottonseed has been carried out at two mills in two locations. Varieties of seed processed and conditions during processing, which included cooking of moistened seed, were essentially the same at both mills. Seeds were also processed at the more southerly mill by screw pressing, for which seed was cooked without added moisture. Crude hydraulic- and screw-pressed oils produced were stored at different temperatures for 10 months, and samples were periodically refined and bleached. Seeds were also stored at the two mills and were periodically processed. Rate of increase of bleach color in oils from stored seed of stored crude oils has been correlated with temperature of storage and with changes in the absorption spectra of the oils.

75. VIX, H.L.E., SPADARO, J. J., WESTBROOK, R. D., CROVETTO, A. J., POLLARD, E. F., and GASTROCK, E. A.

PRE-PILOT-PLANT MIXED-SOLVENT FLOTATION PROCESS FOR SEPARATING PIGMENT GLANDS FROM COTTONSEED MEATS. Amer. Oil Chem. Soc. Jour. 24(7): 228-236. 1947.

This investigation was undertaken to develop on a pre-pilot-plant scale method for separating flaked cottonseed into hulls, meal, and pigment glands. Engineering and technical information is presented relative to fractionation of undefatted as compared to defatted flakes; means of sufficiently disintegrating flakes; selection of suitable solvents; effects of moisture in both solvents and flakes; purification of pigment glands and meal, and yields of purified products. Pigment glands of high purity and essentially gland-free meal in quantities sufficient to permit utilization research were obtained.

76. WILLIAMS, P. A., HADDEN, R. P., [i.e. HADDON, R. P.], HALL, C. M., CASTILLON, L. E., GUICE, W. A., O'CONNOR, R. T., and BOATNER, C. H.

PROCESSING OF COTTONSEED. IV. EFFECT OF PREPARATION AND COOKING OF MEATS ON THE BLEACH COLOR AND STORAGE PROPERTIES OF SCREW-PRESSED OILS. Amer. Oil Chem. Soc. Jour. 26(1): 28-34. 1949.

To study effects on the characteristics of cottonseed meal and oil produced by screw-pressing, conditions were varied with respect to preparation of meats, addition of water, and the temperature and duration of cooking. In laboratory-scale experiments, 10 percent of water added to the flaked meats before cooking at 235° to 244°F. for 1-1/2 hours resulted in a low bleach color in expressed oils. Mill-scale tests indicated that cooking in the presence of relatively large amounts of water improves the storage properties of oil but not its initial bleach color. On the other hand, unwetted rolled or ground meats cooked at a low temperature resulted in low initial bleach color of the oil with very little reversion during storage at room temperature. Widely varying amounts of gossypol were found in both oils and meals produced from meats cooked under various conditions.

77. VIX, H.L.E., SPADARO, J. J., MURPHEY, C. H., JR., PERSELL, R. M., POLLARD, E. F., and GASTROCK, E. A.

PILOT-PLANT FRACTIONATION OF COTTONSEED.
II. DIFFERENTIAL SETTLING. Amer. Oil Chem. Soc. Jour. 26(10): 526-530. 1949.

A new fractionation process termed "differential settling" has been developed to produce a cottonseed meal substantially free of oil, pigment glands, and hulls from either defatted or undefatted flakes. Work on this process was initiated during research on a mixed solvent flotation method of fractionation, which showed several inherent disadvantages, but which produced cottonseed meal essentially free of pigment glands (gossypol content as low as 0.006 percent). Such meal has high nutritional value and excellent potentialities for industrial use. Meal produced by the differential settling process has as low a gossypol content as the meal produced by the flotation principle and overcomes the disadvantages. Two methods, centrifugal and tube settling, have been developed which show promise for commercial use in combination with present solvent-extraction processes.

78. HADDON, R., SCHWARTZ, A. K., WILLIAMS, P. A., THURBER, F. H., KARON, M. L., DECHARY, J., GUICE, W., KUPPERMAN, R., O'CONNOR, R. and ALTSCHUL, A. M.

EFFECT OF PROCESSING CONDITIONS ON THE CHEMICAL PROPERTIES OF COTTONSEED MEALS. Cotton Gin and Oil Mill Press 52 [i.e. 51] (9): 18-20. 1950.

As part of research on improving the nutritional value of cottonseed meal by means of improving the present methods of processing, the changes in chemical properties of cottonseed meals caused by variation in conditions of hydraulic- and screw-press operation were investigated. The processing experiments were conducted in a commercial mill on the effect of conditions of cooking the meats and of the degree of pressure exerted in the screw-press. Changes taking place in the cottonseed protein were measured by determination of nitrogen solubility. This disposition of gossypol and gossypol-like compounds was determined by measurement of content of free-gossypol-like materials in the meal. In screw-press operation it was possible to obtain meals whose analysis showed low gossypol contents with 40 percent of their nitrogen soluble in half molar salt solution. Collaborative feeding tests are under way

for the purpose of correlating laboratory data on these properties with nutritive properties.

79. REUTHER, C. G., JR., SPADARO, J. J., and GASTROCK, E. A.

RESEARCH ON FRACTIONATION OF COTTONSEED MEATS. Cotton Gin and Oil Mill Press 52 [i.e. 51] (2): 12, 35, 39-40. 1950.

Progress in the development of a process of fractionation of cottonseed meats is reviewed, with emphasis upon the contribution of the NCPA Fellow (Reuther) to those investigations. The fractionation process has been improved to make possible increased yields of fine meal, essentially free of pigment glands. Feed flakes suitable for partial solvent extraction prior to fractionation have been produced and the quantities of products necessary for larger-scale evaluation have been made. In the fractionation process, either the defatted or undefatted flakes are disintegrated in a solvent slurry to detach the meal tissue from the pigment glands; the disintegrated solids are then separated either by the gravity flotation method or by the differential settling method; and the solids fractions then recovered from the oil-solvent mixtures (miscellas), which are, in turn, evaporated to recover the oil and solvents. Before the process can be considered for commercial adoption, feed flake preparation, disintegration and screening procedures, and product evaluation must be further investigated.

80. BATSON, D. M., THURBER, F. H., and ALTSCHUL, A. M.

THE EFFECT OF SCREW-PRESS AND HYDRAULIC-PRESS PROCESSING CONDITIONS ON PIGMENT GLANDS IN COTTONSEED. Amer. Oil Chem. Soc. Jour. 28(11): 468-472. 1951.

Contents of intact pigment glands, free gossypol, and total gossypol in uncooked and cooked cottonseed meats, and in press cake samples from laboratory tests, as well as from hydraulic-press and screw-press mills, were determined. In the screw-press mill, where cooking was carried out without adding moisture, recoverable glands or free gossypol changed little during cooking, but were reduced to low levels during passage of the cooked meats through the press. In laboratory tests, when meats which had been cooked at low-moisture content, were subjected to hydraulic pressures of 2,000 and 20,000 p.s.i. recoverable glands were reduced, but free gossypol was not. Wet cooking

of meats decreased free gossypol and intact glands and, although hydraulic pressing failed to further reduce free gossypol, recoverable glands were sharply reduced by pressing. It is suggested that the effectiveness of the screw-press in rupturing and disintegrating pigment glands is due to the development of shearing forces in combination with the compressive-type pressure. It is believed that a shearing action is more effective than compressive force of similar magnitude.

81. KNOEPFLER, N. B., GRACI, A. V., JR., SPADARO, J. J., and GASTROCK, E. A.
RESEARCH ON FRACTIONATION OF COTTONSEED MEATS. Cotton Gin and Oil Mill Press 52(4): 16, 39-42; Oil Mill Gazetteer 55(8): 66-71. 1951.

Progress in the development of a process of fractionation of cottonseed has been made during the past year and is reported. Advances were made in developing continuous pilot-plant unit operations to replace batch operations. Preparation factors affecting fractionation methods of obtaining greater reduction of gossypol were further investigated. Products from fractionation are being evaluated.

82. KNOEPFLER, N. B., VIX, H. L. E., and THURBER, F. H.
RESEARCH ON PROCESSING PROCEDURES TO IMPROVE THE NUTRITIVE VALUE OF COTTONSEED MEAL. Cotton Gin and Oil Mill Press 53(6): 16, 18, 61-66; Oil Mill Gazetteer 56(11): 29-35. 1952.

Progress has been made in an investigation of the processing conditions that would be suitable for production of cottonseed meals of high nutritive value by fractionation, screw-pressing, hydraulic pressing, and solvent extraction. Problem is to remove or inactivate gossypol and similar components without the use of high-temperature cooking. Meals prepared by screw-press mills through procedures in which the temperatures during cooking were 200°F. had a much higher nutritive value than those cooked at 240°, and the latter were much better than those cooked at 280°. Gossypol content of oils produced by low-temperature, dry-cooking screw-press procedures was 1.0 percent as compared with 0.3 to 0.4 percent in the oil from high-temperature processing. An excellent grade of oil was produced by immediate refining and bleaching, but after storage of the crude oil at elevated temperatures the color of the refined oil was increased. Hydraulic-pressed meals having chemical properties that would

indicate a high nutritive value were prepared in the pilot plant by severe rolling of the moistened flakes followed by low-temperature, moist-cooking, drying, and pressing. Butanone-extracted meals have been prepared in the pilot plant as a standard in feeding tests with other meals in nutrition laboratories.

83. EAVES, P. H., MOLAISSON, L. J., BLACK, C. L., CROVETTO, A. J., and D'AQUIN, E. L.
A COMPARISON OF FIVE COMMERCIAL SOLVENTS FOR EXTRACTION OF COTTONSEED. Amer. Oil Chem. Soc. Jour. 29(3): 88-94. 1952.

In pilot-plant-scale batch extraction of cottonseed, a comparison was made of benzene, ethylether, acetone, and butanone of commercial grade with commercial hexane as to effects on yield, composition, properties, and processing characteristics of the meals and the crude oils produced. None of the experimental solvents may be said to compare favorably with hexane, since any improvement in meal quality was achieved at the expense of oil quality. The differences in yields were not such as to offset this loss in oil quality.

84. GRACI, A. V., JR., GARDNER, H. K., CUCULLU, A. F., CROVETTO, A. J., JR., SPADARO, J. J., and KNOEPFLER, N. B.
PILOT PLANT DESOLVENTIZATION OF FINE COTTONSEED MEAL. Amer. Oil Chem. Soc. Jour. 29(2): 41-43. 1952.

Fine cottonseed meal, 300-mesh, was desolventized satisfactorily in a continuous pilot-plant meal dryer of the screw-conveyor type. The free gossypol content of the fine meal was lowered as much as 69 percent during the drying operation without appreciably affecting the protein solubility of the meal. A meal in granular form could be obtained, provided the feed material had certain physical characteristics.

85. REUTHER, C. G., JR., LE BLANC, M.F.H., JR., BATSON, D. M., and KNOEPFLER, N. B.
A PRELIMINARY STUDY OF THE EFFECT OF MOISTURE CONTENT, ROLLING, AND COOKING OF COTTONSEED MEALS ON THE CHEMICAL PROPERTIES OF HYDRAULIC-PRESSED MEALS. Amer. Oil Chem. Soc. Jour. 30(1): 28-32. 1953.

Closely set 5-high rolls, corrugated rolls, and smooth rolls were used to accomplish gland breakage in cottonseed meals, and their effectiveness was compared. The

degree of gland breakage was determined by an empirical method using hexane extraction. Severe (5-high rolls) conditions resulted in a large amount of gland breakage. A maximum gland breakage of 54 percent was generally obtained at moisture contents of 14 percent or above in the meats. Samples having a larger amount of gland breakage had a much lower content of free gossypol after cooking and pressing. Under the best conditions of rolling and cooking, in which the temperature of cooking did not exceed 225°F., a hydraulic-press meal was obtained with a free gossypol content of 0.03 percent and nitrogen solubility of 40 percent.

86. PONS, W. A., JR., MURRAY, M. D., LE BLANC, M.F.H., JR., and CASTILLON, L. E. .
GOSSYPOL MATERIAL BALANCE, DENATURATION OF PROTEIN, AND LOSS OF THIAMINE IN COMMERCIAL PROCESSING OF COTTONSEED. Amer. Oil Chem. Soc. Jour. 30(3): 128-132. 1953.

Cottonseed processed by 5 commercial mills was systematically examined with reference to free gossypol reduction, nitrogen solubility, thiamine reduction, material balances of total gossypol, and the distribution of gossypol in processing. One hydraulic mill reduced free gossypol in meal to a low level, about that obtained in screw pressing. For a given mill, free gossypol contents of meals were fairly uniform. A relatively small amount of total gossypol was lost or destroyed during preparation of meats for either hydraulic- or screw-pressing; no significant loss could be attributed to pressing operations. Screw-pressed oils contained several times as much gossypol as hydraulic-pressed oils, the amount depending on the extent of binding of gossypol in cooking and mechanical preparation of meats. The high temperatures developed in screw pressing contributed to a greater reduction in thiamine and nitrogen solubility than was observed for hydraulic pressing.

87. KNOEPFLER, N. B., EAVES, P. H., AND VIX, H.L.E.
PREPARATION OF COTTONSEED MEATS FOR FILTRATION-EXTRACTION. Cotton Gin and Oil Mill Press 54(6): 98-100. 1953.

Cottonseed meals produced experimentally by different methods of processing are being used in various nutritional investigations. Conditions used in the rolling and cooking operations to prepare cottonseed meats for filtration-extraction have been studied in relation to the

quality of the final meal and to the yield and quality of the oil. The preparation of cottonseed meats for filtration-extraction was compared with that for hydraulic pressing.

88. D'AQUIN, E. L., VIX, H.L.E., SPADARO, J. J., GRACI, A. V., JR., EAVES, P. H., REUTHER, C. G., JR., MOLAISON, L. J., McCOURTNEY, E. J., CROVETTO, A. J., AND GASTROCK, E. A.
FILTRATION-EXTRACTION OF COTTONSEED.
Indus. and Engin. Chem. 45(1): 247-254.
1953.

The pilot-plant development of filtration-extraction is described and data on pilot-plant runs are reported. This simplified solvent process overcomes most problems of direct solvent extraction and is applicable to small- and medium-size mills.

89. THURBER, F. H., VIX, H.L.E., PONS, W. A., JR., CROVETTO, A. J., and KNOEPFLER, N. B.
THE EFFECT OF PROCESSING CONDITIONS ON THE PROPERTIES OF SCREW-PRESS COTTONSEED MEAL AND OIL. Amer. Oil Chem. Soc. Jour. 31(9): 384-388. 1954.

Processing conditions, particularly cooking procedures, markedly influence the chemical properties of screw-pressed meal and oil. Cooking prepared-meats at 240°-250°F., as in normal mill practice, produced meals with low free-gossypol content but at the expense of considerable protein damage. The resultant crude oils showed some color reversion upon storage at 95°F. Dry cooking (7 percent moisture) at temperatures not exceeding 200°F. gave meals of improved chemical properties, but the crude oils exhibited considerable color reversion on storage. Wet low-temperature cooking (200°-210°F.), followed by evaporative cooling, yielded a meal intermediate in quality between that for normal mill practice and dry low-temperature cooking. The crude oils, which correspond to hydraulic-pressed oil, did not exhibit any appreciable color reversion upon storage at elevated temperatures. The selection of processing conditions, notably cooking, enables wide variations in the distribution of gossypol between meal and oil. The increase in the bleach color of crude oils stored at 95°-100°F. was found to be directly related to the initial gossypol content of the crude oils.

90. CANNON, P. R.

RAT REPLETION METHOD. In "Methods for Evaluation of Nutritional Adequacy and Status," ed. by H. Spector and M. S. Peterson. U. S. Quartermaster Food and Container Inst., Surveys of Progress on Military Subsistence Problems, Ser. II, No. 2: 29-38. 1954.

Protein adequacy of cottonseed meal from the Southern Regional Research Laboratory was determined. The results indicated a definite correlation between repletion performance and the varied conditions of processing and cooking of the meals in the extraction process. Extensive cooking, particularly by steam, under pressure causes deterioration in protein values, especially at temperatures above 200°F. (p. 33).

91. PONS, W. A., JR., THURBER, F. H., and HOFFPAUIR, C. L.

PREPRESS-SOLVENT EXTRACTION OF COTTON-SEED, PROCESSING CONDITIONS AND CHARACTERISTICS OF PRODUCTS. Amer. Oil Chem. Soc. Jour. 32(2): 98-103. 1955.

A study has been made of the relation between processing conditions and the chemical characteristics of cottonseed meals and oils produced by prepressing-solvent extraction. Twenty-six complete sets of mill samples of known processing history and representative of the production at 11 mills were used in the investigation.

Cooking conditions were the major factor influencing the distribution of the gossypol between the meal and the oil. Reduction in free gossypol during cooking was due to binding with meal components while that occurring during prepressing and solvent extraction resulted mainly from removal of gossypol in the prepressed and solvent-extracted oils.

Nitrogen solubility data, which have been suggested as a measure of protein damage, indicated that the major change or reduction in nitrogen solubility occurred during cooking. Very little reduction was noted for prepressing or solvent extraction. The reduction in nitrogen solubility during prepressing is much smaller than that previously reported for normal screw-pressing operations.

Prepressed oils gave lower refining losses and lower refined and bleached color than did the solvent-extracted oils. Bleach color reversion, after storage of crude

oils for 30 days at 100°F., was greater for solvent-extracted than for prepressed oils.

A number of meals exhibited the desirable characteristics of low free gossypol content and high nitrogen solubility. Values calculated for chemical indexes of protein quality (See Item 102) indicate that many of the meals should have good protein quality.

92. CHANG, W.-Y., COUCH, J. R., LYMAN, C. M., HUNTER, W. L., ENTWISTLE, V. P., GREEN, W. C., WATTS, A. B., POPE, C. W., CABELL, C. A., and EARLE, I. P.

THE NUTRITIONAL VALUE OF PREPRESS-SOLVENT COTTONSEED MEALS. Amer. Oil Chem. Soc. Jour. 32(2): 103-109. 1955.

Four separate laboratories independently conducted feeding studies in which each laboratory employed the type test of its choice on 26 complete sets of prepressed-solvent extracted cottonseed meal samples obtained from 11 commercial mills and one standard, solvent extracted cottonseed meal prepared at Southern Regional Research Laboratory. The processing history and the chemical characteristics of these meals were previously determined and known. (See Item 91).

The purpose of these coordinated feeding studies was to evaluate further the relationship of the chemical and physical characteristics of the cottonseed meals to the nutritional value of the protein.

Despite differences of animals and the type nutritional tests employed, all the laboratories are in good agreement that results of chemical analyses and chemical index values are reliable in predicting results of actual feeding studies.

The correlation coefficients obtained by all four laboratories indicate that nitrogen solubility with 0.02 N NaOH is a better measure of protein quality than dilute NaCl; also, that with these prepress-solvent extracted cottonseed meals, total gossypol content and nitrogen solubility in 0.02 N NaOH have approximately equal value as indicators of protein quality. Further investigations are to be made on the relationship of total gossypol to protein quality.

If meals are produced with high nitrogen solubility and low free and total gossypol contents, these meals should be high in nutritive value.

93. STANSBURY, M. F.
FREE GOSSYPOL AND PROTEIN SOLUBILITY. Oil
Mill Gazetteer 60(4): 29-30. 1955.

Free gossypol contents and nitrogen solubilities of a limited number of samples of fine types of cottonseed meals are reported: hydraulic press; normal screw-press; low-temperature screw-press; prepress, solvent-extraction; and filtration.

Conditions imposed by prepress solvent-extraction and by low-temperature screw-pressing appear to contribute on the average to the production of meals containing low levels of free gossypol and having high nitrogen solubilities.

94. GRAU, C. R., and ZWEIGART, P. A.
THE EFFECTS OF VARIOUS PROCESSING METHODS
ON THE VALUE OF COTTONSEED MEAL AS AN
AMINO ACID SOURCE FOR CHICKENS. Poultry Sci.
34(3): 724-728. 1955.

Cottonseed meals of high protein quality were found among samples produced by hydraulic, screw-press, or prepress solvent methods. Use of any one of these methods did not, of itself, guarantee high quality. In general, the best meals were produced at the lowest temperatures.

Most of the meals were produced under the close supervision of workers from the Southern Utilization Research Branch (U.S.D.A.)

95. CONLY, L. J., POPE, C. W., and WATTS, A. B.
THE EFFECT OF pH DURING PROCESSING UPON
THE NUTRITIVE VALUE OF COTTONSEED MEAL.
Poultry Sci. 34(5): 1231. 1955.

The effect of pH during processing on the nutritive value of cottonseed meal was investigated in studies involving 2,960 chicks in 10 feeding trials. These tests involved feeding the cottonseed meals in rations containing both optimum and sub-optimum levels of protein. These meals were prepared over a wide range of pH values and extensive tests were conducted using rations of sub-optimum protein level to establish the relationship between the pH of the prepared meal and its nutritive value. From the results of these low level protein quality evaluations, sets of conditions were selected and larger samples of meals were prepared for testing under practical ration conditions.

A cottonseed meal prepared at pH 8.2 proved to be slightly superior to two excellent commercially prepared cottonseed meals. This particular meal was equal to soybean oil meal as a source of supplementary protein in practical broiler rations.

96. HOPPER, T. H.

INFLUENCE OF COMMERCIAL PROCESSING CONDITIONS ON PROPERTIES OF COTTONSEED PRODUCTS. Oil Mill Gazetteer 60(3): 14, 16-17. 1955.

The influence of conditions imposed by the several commercial methods of processing cottonseed on the properties of cottonseed oil and meal are reviewed briefly. The variables discussed are composition of kernels and meal, properties of oil, and processing methods. Comparative data on the influence of types of processing methods on the free gossypol content and nitrogen solubility of some cottonseed meals are summarized.

97. GASTROCK, E. A., VIX, H. L. E., and D'AQUIN, E. L.
PREPARATION OF MEATS AND PROCESSING CONTROLS FOR LOW FREE GOSSYPOL AND HIGH PROTEIN SOLUBILITY. Oil Mill Gazetteer 60(5): 12-13. 1955.

Free gossypol content and protein solubility in dilute alkali are two chemical indices used to measure or rate the toxicity and nutritive value of cottonseed meals. This paper is a review of various unit operations in cottonseed processing in order to show how they affect the free gossypol content and alkali solubility. Aside from general conditions, factors discussed are storage, conditioning and tempering, rolling or flaking, cooking, oil extraction, solventizing, toasting, degossypolization.

SEE ALSO: 1, 2, 3, 4, 6, 7, 8, 9, 11, 13, 16, 117, 123, 124, 129, 132, 133.

V. PROCESSING

Effect on Protein

98. KARON, M. L., ADAMS, M. E., and ALTSCHUL, A. M.
ELECTROPHORETIC ANALYSIS OF PEANUT AND
COTTONSEED MEALS AND PROTEINS. Jour. Phys.
& Colloid. Chem. 54(1): 56-66. 1950.

Cottonseed and peanut meals and the derived proteins were analyzed by means of the Tiselius electrophoresis apparatus. The effect of (1) method of extraction of the protein and (2) of the buffer and pH of the buffer solutions was investigated. By fractional precipitation involving a change of ionic strength the two major components of cottonseed protein can be concentrated to over 80 percent purity. Approximately 75 percent of peanut protein consists of two components. These remove soluble sugars and phytin, in which case the two major components separate into two almost equal fractions.

99. INGRAM, G. R., CRAVENS, W. W., and ELVEHJEM, C. A.
EVALUATING COTTONSEED MEAL PROTEIN FOR
CHICK GROWTH BY ENZYMATIC RELEASE OF
AMINO ACIDS. Poultry Sci. 29(4): 590-594. 1950.

A study has been made of the liberation of certain amino acids by acid and enzymatic hydrolysis of cottonseed meal. The enzymatic release of these amino acids from the protein has been correlated with the growth of chicks fed the corresponding samples.

The results show that there is good correlation between the release of certain amino acids by the in vitro technique employed in these studies and the growth supported in chicks by the cottonseed meal. The toxic substances present in certain samples did not alter the results of this method of testing the biological value of the cottonseed meal.

00. KARON, M. L., ADAMS, M. E., and NEWMAN, S.
THE EFFECT OF TEMPERATURE ON THE ELECTROPHORETIC ANALYSIS OF COTTONSEED MEAL EXTRACTS. Jour. Colloid Sci. 7(4): 407-413. 1952.

The electrophoretic patterns of the protein material extracted from a cottonseed meal in the ethylamine barbitol buffer have been investigated over the temperature range of 0° to 20°C. The results indicated no

changes in relative concentrations of the five components as a function of temperature. The application of a single viscosity correction to the solvent could not entirely compensate for the change in mobility of each component with temperature. The viscosity-mobility product displayed a decrease with increasing temperature, but this trend varied irregularly for the several components present.

101. HORN, M. J., BLUM, A. E., WOMACK, M., and GERSDORFF, C.E.F.

NUTRITIONAL EVALUATION OF FOOD PROTEINS BY MEASURING AVAILABILITY OF AMINO ACIDS TO MICROORGANISMS. I. COTTONSEED PROTEINS. Jour. Nutrition 48(2): 231-241. 1952.

Two methods of evaluating the nutritional value of processed cottonseed meals were used. (1) The rat assay method showed that the nutritional value for the rat was considerably altered by cooking the cottonseed at various times, temperatures, and moisture contents. (2) The microbiological method showed that the changes in nutritional value were due to a change in the availability of amino acids. Different methods of processing affected availability of different amino acids. Comparison of the results from assays of acid and enzyme hydrolysates by the microbiological method showed that, although the amino acids were present, some of them were bound in such a way that they were no longer available to the microorganism. The assay of acid hydrolysates showed that only very severe temperatures and pressures caused any destruction of the amino acids. An enzyme digestion system was presented which appears to give good reproducibility of results; the availability of the digestion products to the microorganisms correlated well with the results of rat feeding.

102. LYMAN, C. M., CHANG, W. Y., AND COUCH, J. R.
EVALUATION OF PROTEIN QUALITY IN COTTONSEED MEALS BY CHICK GROWTH AND BY A CHEMICAL INDEX METHOD. Jour. Nutrition 49(4): 679-690. 1953.

Twenty-three samples of cottonseed meals, made by different manufacturing processes, were evaluated by chick feeding tests. Wide variations in the protein quality of the meals made by each process were found. Low temperatures in processing did not always produce high quality meals. Although the free gossypol content of most of the meals was quite low, the total gossypol content of the meals proved to be an important

factor in determining the nutritional value of the protein. A relationship was found between the solubility of the protein in 0.02N sodium hydroxide and the nutritional value of the meal. A chemical index which takes into account both total gossypol content and nitrogen solubility, in 0.02N sodium hydroxide, was devised for the chemical evaluation of protein quality in cottonseed meals. A good correlation was found between the chemical index values and chick growth rate. Lysine supplementation of the poorer meals resulted in an increased growth rate of chicks amounting to over 100 percent. Lysine supplementation of the better meals resulted in some improvement in growth rate, but the percentage increase was less.

103. KARON, M. L., ADAMS, M. E., and ALTSCHUL, A. M.
COTTONSEED MEAL EXTRACTS: ELECTROPHORETIC PATTERNS OF BUFFER EXTRACTS OF DIFFERENT NUTRITIVE VALUE. Jour. Agr. and Food Chem. 1(4): 314-318. 1953.

To determine the nature of changes taking place in cottonseed meal proteins as a result of differences in conditions of preparation, the electrophoretic patterns of cottonseed meal extracts were studied. (This is part of a research program on the improvement of the nutritional value of cottonseed meal and the laboratory determination of differences in nutritive value of the meal as a protein supplement.) As heat stress during processing increased, electrophoresis patterns of the soluble protein fraction of the meal changed. A new fastmoving component developed, and the original two major components became more difficult to resolve. These changes seem to correlate well with changes in nutritive value within a single series of related meals.

104. KUIKEN, K. A.
AVAILABILITY OF THE ESSENTIAL AMINO ACIDS IN COTTONSEED MEAL. Jour. Nutrition 46(1): 13-25. 1952.

The availability of the essential amino acids in cottonseed meal samples which were processed in various ways was determined by a rat feeding method. Marked variation in individual amino acid availability was characteristic of commercial hydraulic cottonseed meal. Lysine and methionine were particularly low, with values of 64 and 67 percent, respectively. Lysine availability values as high as 85 percent were obtained with special solvent and gland-free meals. The binding of gossypol as a result of heating cottonseed meal in the presence of either gossypol or pigment glands did not reduce amino acid availabilities appreciably unless

cottonseed oil was also present. Heat treatment as severe as autoclaving for one hour at 15 lbs. pressure did not reduce the availability of the essential amino acids in cottonseed meal of low oil content. Lysine was more sensitive to heat destruction than the other essential amino acids. About a 10 percent reduction in lysine content was observed when solvent-processed cottonseed meal was autoclaved at 15 lbs. pressure for 60 minutes.

105. JENSEN, E. A., CONDON, M. Z., KARON, M. L., and
ALTSCHUL, A. M.
RESEARCH ON COTTONSEED MEALS. Cotton Gin
and Oil Mill Press 54(5): 24-25, 28. 1953.

An attempt to develop a chemical measure of the nutritive value of cottonseed meal is part of a cooperative program of research on cottonseed processing to obtain oil of high quality with meal of improved nutritive value. Changes in chemical and physical properties of a cottonseed meal of known high nutritive value as the result of autoclaving have been investigated. There is no change in the total or acid-soluble phosphorus or total nitrogen content; but free gossypol content and protein solubility in sodium chloride after autoclaving decrease sharply. Inorganic phosphorus gradually increases as time of autoclaving increases. Protein solubility in sodium hydroxide, total gossypol content, and soluble carbohydrate content decrease as time of autoclaving increases. Electrophoretic patterns indicate progressive degradation of protein as time of autoclaving increases. Meals will be autoclaved in the presence of gossypol alone, in the presence of oil alone, and in the presence of both oil and gossypol. The effects of dry heat on the meal will also be studied. The properties of the meals whose nutritive values have been lowered by the various methods will then be compared. Special emphasis will be placed on the changes in the properties of the protein fractions, since cottonseed meal is sold as a protein supplement. A chemical test of nutritive value based on differences in protein properties obtained in these laboratory experiments should be applicable to commercial meals.

106. SURE, B., with the assistance of EASTERLING, L.,
DOWELL, J., and CRUDUP, M.
COTTONSEED MEALS: INFLUENCE OF PROCESS-
ING ON PROTEIN VALUES. Jour. Agr. and Food
Chem. 1(1): 82-84. 1953.

A biological study was made of the protein values of three cottonseed meals and one cottonseed flour, as

influenced by different methods of processing, using the albino rat as the experimental animal, with the objective of obtaining a meal of low free gossypol content and high biological value. The protein efficiency varied inversely with the amount of heat treatment. The highest and lowest values were obtained with the solvent-extracted (butanone) meal and the cottonseed flour, which were the least and most drastically heated, respectively. The two intermediate meals were low temperature (200°F.) and high temperature (230°F.) screw-pressed meals. Improvement of the biological value of the proteins in the cottonseed flour by addition of lysine and methionine indicated that they were partially destroyed or made unavailable during processing. The results of these studies may stimulate the commercial production of cottonseed meals and cottonseed flours of higher biological values by modification of methods of processing.

107. WOMACK, M., MARSHALL, M. W., and SUMMERS, J. C.
COTTONSEED FOOD SUPPLEMENT: NUTRITIVE
VALUE OF BREAD AND COOKIES CONTAINING
COTTONSEED FLOUR. Jour. Agr. and Food Chem.
2(3): 138-140. 1954.

Water bread containing 10 parts of cottonseed flour per 100 parts of wheat flour (white) gave significantly higher rates of gain per gram of nitrogen consumed than water bread without the cottonseed flour when fed to young rats at levels furnishing 10% protein. When the effect of the addition of cottonseed flour containing nonfat milk solids was tested, it was found that no significant increase in the nitrogen efficiencies was brought about by the cottonseed flour when the breads were both fed at the same protein level. However, when the breads were fed at the same percentage by weight, the higher protein content of bread containing cottonseed flour brought about significantly higher rates of gain than the same amount of bread without cottonseed flour. No significant results were obtained with the cookies.

108. CABELL, C. A., and EARLE, I. P.
PROTEIN QUALITY ASSAY: COMPARISON OF THE
RAT REPLETION METHOD WITH OTHER METHODS
OF ASSAYING THE NUTRITIVE VALUE OF PRO-
TEINS IN COTTONSEED MEALS. Jour. Agr. and
Food Chem. 2(15): 787-790. 1954.

Data are reported from the application of a rat repletion method on estimation of protein quality in 12 cottonseed

meals used in a collaborative study of the effects of processing methods on the nutritive properties of cottonseed meal. Mean gain in body weight by groups of protein depleted rats during a 10-day repletion period was used as the criterion of response for estimating protein quality.

Results with the rat repletion method are correlated with those obtained on the same samples by other laboratories using other biological methods and with other constants on the meals. The rat repletion method is shown to be helpful in measuring protein quality in meals.

109. HORN, M. J., BLUM, A. E., and WOMACK, M.
AVAILABILITY OF AMINO ACIDS TO MICRO-ORGANISMS. II. A RAPID MICROBIAL METHOD OF DETERMINING PROTEIN VALUE. Jour. Nutrition 52(3): 375-381. 1954.

A rapid method for determining the nutritive values of processed cottonseeds has been developed by making a microbiological assay employing Leuconostoc mesenteroides P-60, and using an enzymatic digest of the meal as the only source of protein in the medium. The enzymatic digests were prepared by treating the meals successively with pepsin, trypsin, and hog mucosa powder. Protein index values, computed from the growth-promoting activities of these digests for L. mesenteroides, were compared with the indexes obtained from rat growth studies. In general, both methods ranked the meals in the same order of nutritive value. Since no amino acids are used in the basal medium, the method is relatively inexpensive and results can be obtained in a short time.

110. CONDON, M. Z., JENSEN, E. A., WATTS, A. B., and POPE, C. W.
NUTRITIONAL VARIATION IN COTTONSEED: EFFECT OF AUTOCLAVING IN PRESENCE AND ABSENCE OF GOSSYPOL ON SOLVENT EXTRACTED COTTONSEED MEAL. Jour. Agr. and Food Chem. 2(16): 822-826. 1954.

Before the processing conditions in cottonseed oilmills can be modified to produce meals of consistently high nutritional quality, the individual effects of heat and bound gossypol must be understood. This investigation was undertaken to determine the effect of moist autoclaving in the presence and absence of gossypol on certain chemical and nutritive properties of a cottonseed meal of initially high quality. Chick-feeding

experiments indicated a progressive decrease in the protein quality index of the meals as time of autoclaving increased. This reduction was paralleled by similar decreases in solubility of the meals in 0.02N sodium hydroxide. There appeared to be no direct relationship between any other property of the meals and their nutritive value to chicks. Samples to which 1 percent gossypol was bound during autoclaving appeared to be equivalent, chemically and nutritionally, to control samples autoclaved for the same time.

SEE ALSO: 6, 7, 12, 78, 90, 91, 92, 94.

VI. FEEDS AND FEEDING

111. WARD, A. L.

COTTONSEED MEAL IN FEEDS. Amer. Feed & Grain 37(10): 52-53, 69. 1953.

In 1952-53 oilseed meals supplied approximately 65 percent of the supplementary protein fed livestock and poultry. The nutritive quality of protein is affected by processing conditions, which are being studied in a research program designed to improve nutritive quality of meals.

A toxic factor, gossypol, in the cottonseed has imposed certain restrictions in the use of meals in respect to the levels it can be fed in rations for swine and poultry. This limitation has necessitated an extensive cooperative research program.

Results obtained by various workers indicate; (1) Cottonseed meal containing no more than 0.03 percent free gossypol can be used in unlimited amounts for growing poultry; (2) Cottonseed meal containing 0.04 percent gossypol as 30 percent of the diet of growing chicks and swine can be safely used, when the lysine content is supplemented; (3) Cottonseed meal can so be prepared as to be used in unrestricted amounts for swine and poultry rations; (4) Values of rations have been increased by using combinations of soybean and cottonseed meals, due to a better balance of amino acids than obtained by either meal singly.

Additional research is needed (1) for production of cottonseed meals which can be used in unrestricted amounts for laying hens, and (2) for producing better meals through controlled processing conditions in addition to a quick laboratory method for the determination of nutritive quality of cottonseed meal for poultry and swine.

112. WAUGH, R. K., WISE, G. H., and HOLLON, B. F. COTTONSEED MEAL FOR DAIRY CALVES. East. Feed Merchant 4(11): 26. 1953.

Cottonseed meal has been regarded as a good protein supplement for mature cows but due to its gossypol content has not been used freely in calf rations.

Workers at North Carolina Experiment Station have begun some preliminary studies on cottonseed meal toxicity in young dairy calves. Using a total of four

diets, abnormally high levels of cottonseed meal were fed in an effort to determine the maximum level that could be fed without producing typical gossypol toxicity symptoms. Two of the four diets contained 60 and 40 percent of a commercially available cottonseed meal, with final abnormally high free-gossypol contents.

The other two diets contained cottonseed meal processed according to specifications developed at Southern Regional Research Laboratory and had lower than average free-gossypol contents. The basal ration was properly balanced, and soybean oil meal was used only in the 40 percent cottonseed meal ration for adjusting total protein content.

Sixteen calves--four to each diet--were used. Typical gossypol symptoms were evident both at 60 and 40 percent high gossypol cottonseed meal diets, and also on the 60 percent low gossypol cottonseed meal diet. Growth rates were also affected adversely on the three diets above. No adverse effect was obtained with the 40 percent low gossypol cottonseed meal diet.

In spite of high levels of cottonseed meal, the diets were surprisingly palatable.

Results obtained do not have practical application as yet, and were intended as preliminary studies for determining how much meal can be used in calf rations, if the level of free gossypol can be reduced.

113. COUCH, J. R.

AN OPPORTUNITY TO LOWER COSTS--COTTON-SEED MEAL FOR POULTRY. Cotton Gin and Oil Mill Press 55(10): 7-8, 59. 1954.

Commercial broiler production offers a tremendous market for cottonseed meal which has been processed so that it is suitable for use in these rations.

Reference is made to the discussion and proposals adopted at the Southern Laboratory's conferences on cottonseed meal processing and to the experiments of many outstanding workers in the field.

SEE ALSO: 3, 7, 10, 15, 16, 19, 33, 64, 92.

VI-A. FEED FOR POULTRY

114. GRAU, C. R.

PROTEIN CONCENTRATES AS AMINO ACID SOURCES FOR THE CHICK: CORN GLUTEN MEAL, COTTONSEED MEAL AND PEANUT MEAL. Jour. Nutrition 32(3): 303-311. 1946.

Diets containing corn gluten meal, cottonseed meal, or peanut meal were fed to young chicks so that all the 20 percent crude protein of each diet was provided by one of these concentrates. Additions of various amino acids were made, and the effects on growth and efficiency of gain were noted.

Corn gluten meal required the addition of arginine, lysine, and tryptophane in order to increase the rate of growth from 2 percent to slightly less than 6 percent per day. Cystine, glycine, methionine, threonine, and valine were already present in adequate amounts.

Supplementation of cottonseed meal with both methionine and lysine increased the growth rate from 4 percent to 7 percent. The methionine deficiency was found to be less marked than that of lysine, a result which was expected from amino acid analyses.

Peanut meal is lacking primarily in methionine, but is also slightly deficient in lysine.

115. GROSCHKE, A. C., RUBIN, M., and BIRD, H. R. GLAND-FREE COTTONSEED MEAL AS A PROTEIN SUPPLEMENT FOR CHICKENS. Poultry Sci. 26(3): 310-312. 1947.

Gland-free cottonseed meal, prepared at SURB, when fed to chicks and hens was superior to commercial cottonseed and soybean meals, judged by either growth or hatchability. Feeding pigment glands with soybean meal had the same detrimental effect on growth as feeding raw cottonseed meal containing an equivalent quantity of glands. Storage experiments with eggs showed that all from hens fed commercial cottonseed meal (source unstated) developed olive yolks and 73 percent developed pink albumen. Of those fed gland-free meal one-third developed olive yolks and none pink albumen.

116. HEYWANG, B. W.

A COMPARISON OF COTTONSEED AND SOYBEAN MEALS IN DIETS FOR LAYING CHICKENS. Poultry Sci. 26(5): 442-446. 1947.

Diets fed to laying White Leghorn pullets contained only enough fish meal or meat scraps to furnish less than 3 percent of animal protein, but also contained 15 percent cottonseed meal, or 15 percent soybean meal, or 7.5 percent cottonseed meal plus 7.5 percent soybean meal. Small amounts of ferrous sulfate were also included in some diets. As measured by relative egg production, feed consumed, body weight, and mortality, cottonseed and soybean meals were about of equal quality. The inclusion of ferrous sulfate decreased egg production in most cases. Feeding hens on a diet containing 20 percent cottonseed meal (0.059 percent "free" and 0.811 percent "bound" gossypol) yielded eggs which exhibited discolored whites and/or yolks after 6 months' storage; 10 percent of the meal in the diet resulted in eggs normal in color.

117. BOATNER, C. H., ALTSCHUL, A. M., IRVING, G. W., JR., POLLARD, E. F., and SCHAEFER, H. C.

THE NUTRITIVE VALUE OF COTTONSEED FOR CHICKS AS AFFECTED BY METHODS OF PROCESSING AND CONTENT OF PIGMENT GLANDS. Poultry Sci. 27(3): 315-328. 1948.

An investigation is reported of the effect on the growth of chicks of uncooked cottonseed extracted with hexane, uncooked cottonseed extracted with diethyl ether, the foregoing solvent-extracted meals subsequently heated, hydraulic-pressed cottonseed meal, hydraulic-pressed cottonseed meal extracted with hexane, gland-free cottonseed flour, gossypol, and cottonseed pigment glands. The cottonseed meals were substituted for soybean meal in a basal diet containing 13 percent of screw-pressed soybean meal as the source of vegetable protein, and the gossypol and pigment glands were added in measured amounts to the basal soybean meal diet. The basal soybean meal diet and the experimental cottonseed diets were fed to parallel lots of 20 to 30 chicks each for a period of six weeks.

Gland-free cottonseed flour; uncooked, diethyl ether-extracted cottonseed; and hydraulic-pressed cottonseed meal supported excellent growth, whereas, markedly inferior growth resulted when the experimental diets containing uncooked, hexane-extracted cottonseed or pigment glands were fed to chicks. These results

showed that all of the physiologically deleterious components of cottonseed are segregated in the pigment glands.

Pure gossypol added to the diet produced relatively little retardation in the growth of chicks, and a poor correlation was found between the nutritional values of the various cottonseed products and their contents of gossypol and gossypurpurin.

118. HEYWANG, B. W., DENTON, C. A., and BIRD, H. R.
THE EFFECT OF THE DIETARY LEVEL OF
COTTONSEED MEAL ON HATCHABILITY. Poultry
Sci. 28(4): 610-617. 1949.

Differences between the hatchability of eggs produced on a basal diet containing no cottonseed derivatives and the hatchability of eggs produced on the other diets were statistically significant only when the diets contained 20 or 30 percent of hydraulic meal (0.016 and 0.024 percent free gossypol), or 20 to 30 percent of a solvent extracted meal (0.024 and 0.036 percent free gossypol), or 3.75 percent of raw cottonseed. If these meals contain no more than 0.12 percent free gossypol, a 10 percent meal level may be included in the diets of breeders with no adverse effect on egg hatchability. Inclusion of sardine meal in the diets did not increase hatchability; inclusion of casein decreased it. After about 4 months of cold storage, most of the eggs produced on the cottonseed diets exhibited yolk discoloration, the degree of darkening increasing as the free gossypol content of the diets increased. Diets containing 20 and 30 percent levels of the hydraulic meal caused the pullets to gain weight; corresponding levels of solvent meals caused weight losses.

119. GRAU, C. R.
COTTONSEED MEAL FOR CHICKS: EXPPELLER-
TYPE MEAL AS PRODUCED IN CALIFORNIA CAN
BE FED AT LEVELS AS HIGH AS 40 PERCENT OF
THE DIET. Calif. Agr. (Calif. Agr. Expt. Sta.) 4(9):
14-15; Coop. Poultryman 15(10): 3-4. 1950.

Expeller-type cottonseed meal can be used extensively for chick-starting, broiler-fryer, and growing rations for chicks to provide the principal source of protein. Such rations should not be fed to laying hens, however, because of their adverse effect upon interior egg quality, particularly after storage. Under practical

conditions, diets such as the ones used in these experiments containing 40 percent cottonseed meal probably need no amino acid supplements.

120. HEYWANG, B. W., and BIRD, H. R.
SUPPLEMENTS FOR COTTONSEED MEAL IN DIETS
FOR CHICKENS. Poultry Sci. 29(4): 486-495. 1950.

Hatchability of the eggs of White Leghorns was increased when 0.8 percent DL-lysine was included in their diet containing 30 percent cottonseed meal, and 5 percent dried cow manure as a source of vitamin B₁₂. It is possible, however, that the lysine functioned in detoxifying the gossypol in the cottonseed meal and not in overcoming an amino acid deficiency. The results of experiments with growing White Leghorn chickens show that cottonseed meal is deficient in vitamin B₁₂ (as found in dried cow manure) and lysine.

121. RICHARDSON, L. R., and BLAYLOCK, L. G.
SUPPLEMENTS TO SOYBEAN AND COTTONSEED
MEAL DIETS FOR POULTS AND GROWING TUR-
KEYS. Poultry Sci. 29(5): 651-655. 1950.

Fish meal, fish solubles, and an APF concentrate were investigated as supplements to a milo-soybean meal diet for poults. The poults which received fish meal or the APF concentrate were larger at 6 weeks than those that received the basal diet or fish solubles, but the differences were not statistically significant. Cottonseed meal was also tested as a source of protein for poults. One diet which contained cerelese and 60 percent of cottonseed meal supported a rapid rate of growth in poults when it was supplemented with 0.5 percent of L-lysine, 0.2 percent of DL-methionine, and 0.1 percent of DL-tryptophan. When a milo-cottonseed meal diet was supplemented with only 0.3 percent of L-lysine, white feathers, severe perosis, and a slow rate of growth were obtained. The rate of growth increased immediately and pigment returned to new feathers when the lysine was increased to a level of 0.8 percent. Mixtures of soybean and cottonseed meals which were tested were unsatisfactory for poults. The mortality rate was high and there was a high incidence of white feathers and of perosis. The absence of pigmentation in feathers suggested that the mixtures were deficient in lysine.

122. HEYWANG, B. W., BIRD, H. R., and ALTSCHUL, A. M.
THE EFFECT OF PURE GOSSYPOL ON EGG HATCH-
ABILITY AND WEIGHT. Poultry Sci. 29(6): 916-920.
1950.

High hatchability was the basis of selecting 10 White Leghorn pullets for each 4 experimental groups for feeding tests. One group was fed the diet with no gossypol and the others the diet in which pure gossypol was mixed at the 0.012, 0.024, and 0.036 percent levels. The mixed diets were fed only on the day of mixing and the following day. Gossypol had an appreciable adverse effect on hatchability and egg weight when it was mixed with the diet at the 0.024 percent level and a still greater adverse effect at the 0.036 percent level, but did not affect either at the 0.012 percent level. Hatchability and egg weight returned to normal after gossypol feeding was stopped.

123. MILLIGAN, J. L., and BIRD, H. R.
EFFECT OF PROCESSING VARIANTS ON THE NU-
TRITIVE VALUE OF COTTONSEED MEAL FOR
CHICKS. Poultry Sci. 30(5): 651-657. 1951.

Variations in either the cooking or pressing of cottonseed meals were found to affect the nutritional value of the meal for growing chicks. Meals processed at a maximum temperature of 200°F. or less were more nutritious than those cooked above 200°F. In general, meals pressed with an energy input of 50 amperes contained more salt-soluble nitrogen and were better nutritionally than those pressed with an energy input of 60 amperes. Cottonseed meals containing 0.01 to 0.108 percent free gossypol were fed safely as 39 percent of the total diet and the only protein supplement. If the meals were fed as 70 percent of the total diet, 0.02 percent free gossypol was not harmful but 0.108 percent was. When the meals were low in free gossypol, chicks attained greater final weights on 70 percent than on 39 percent cottonseed meal in the diet. These experiments indicated that it may be possible to process cottonseed meal so that it would equal soybean meal in feeding value for growing chicks.

124. GERMAN, H. L., and COUCH, J. R.
EFFECT OF METHODS OF PROCESSING ON THE
NUTRITIVE VALUE OF COTTONSEED MEAL FOR
CHICKS. Poultry Sci. (Abstract) 30(6): 913. 1951.

Straight-run New Hampshire chicks used in these studies were maintained in batteries with raised screen floors.

Four samples of cottonseed meal processed by different methods were supplemented in a basal diet, which was composed of 60 percent yellow corn and milo maize, 35 percent soybean oil meal, 3 percent dehydrated alfalfa leaf meal, 0.5 percent vitamin B₁₂ aureomycin concentrate, plus minerals and vitamins.

The results show that the method of processing affected the nutritive value of the samples. The response in terms of body weight and feed efficiency at 10 weeks of age, obtained when supplementing samples with DL-lysine and dried whey and fish meal, showed that amino acids were probably made more available and the gossypol less available.

125. HEYWANG, B. W., BIRD, H. R., and KUPPERMAN, R. P.

THE LOSS OR INACTIVATION OF PURE GOSSYPOL IN A MIXED DIET. Poultry Sci. 31(1): 35-39. 1952.

Most of the gossypol mixed with a diet (for poultry) at the 0.012, 0.024, and 0.036 percent levels was undetectable by chemical analysis after the mixtures had stood for several days. To learn whether the gossypol had been destroyed or rendered inactive for chickens, a 42-day experiment was conducted with four groups, each containing 10 White Leghorn pullets--selected because of the high hatchability of their eggs when all had been fed the basal diet containing no gossypol. During the 42 days, the basal diet contained pure gossypol mixed at the 0, 0.012, 0.024, or 0.036 percent levels. The mixed diets were fed on the sixth and seventh days after mixing. There was no decrease in hatchability with gossypol at the 0.012 and 0.024 percent levels, and only a slight decrease at the 0.036 percent level. There was no adverse effect on whole egg weight at any of the three levels.

126. STEPHENSON, E. L., and SMITH, R. M.

THE STORAGE QUALITY OF EGGS PRODUCED BY HENS FED SCREW-PRESSED COTTONSEED MEAL. Poultry Sci. 31(1): 98-100. 1952.

Cottonseed meal is an important protein supplement in animal feeds. However, it is not generally recommended for use in diets of laying hens due to a development of egg yolk and albumen discoloration; the former attributed to gossypol content and the latter, obtained upon storage, attributed to some unidentified substance other than gossypol in cottonseed meal.

A feeding study was conducted on three groups of laying hens, in which three different meals were incorporated for protein supplement: 1. Commercial solvent-extracted cottonseed meal, gossypol content 0.61 percent; 2. Laboratory (Southern Regional Research Laboratory) processed screw-pressed cottonseed meal, gossypol content 0.01 percent; and 3. Soybean meal, as control.

After 10 days of feeding the experimental diets elapsed, the eggs were collected and stored for 6 months and were graded by U. S. Standards, then broken and examined visually.

Data obtained indicated that on the basis of market quality, eggs produced by the hens fed the screw-pressed cottonseed meal used in this experiment was satisfactory for incorporation in a poultry laying ration as the principal source of protein. The storage quality of the eggs produced from the hens fed the screw-pressed cottonseed meal was similar to those obtained from the hens receiving soybean oil meal. The eggs produced by the hens fed the commercial solvent-extracted cottonseed meal had the lowest market value, with 100 percent discoloration of yolks, 70 percent pink discoloration of albumen, and 50 percent poor storage or keeping quality.

127. MACHLIN, L. J., DENTON, C. A., and BIRD, H. R. SUPPLEMENTATION WITH VITAMIN B₁₂ AND AMINO ACIDS OF CHICK DIETS CONTAINING SOYBEAN OR COTTONSEED MEAL. Poultry Sci. 31(1): 110-114. 1952.

Day-old Rhode Island Red chicks were fed test rations until 6 weeks of age, and records of weights, mortality, feed consumption and deficiency symptoms were kept. Methionine was the only amino acid deficient in a simple corn-soybean meal diet, and further supplementation with Vitamin B₁₂ was necessary for maximum growth response from this diet. Lysine was the only amino acid deficient in a corn-cottonseed meal basal diet, but the further addition of Vitamin B₁₂ generally failed to cause increase in growth. When both lysine and an aureomycin fermentation product were added, growth was better than that produced by a good commercial ration. The cottonseed meal basal contained 34 percent of a hydraulic cottonseed meal, while the soybean meal basal contained 35 percent of an expeller process soybean meal.

128. HEYWANG, B. W., and BIRD, H. R.

FAILURE OF SEVERAL AMINO COMPOUNDS AND OF SARDINE MEAL TO INACTIVATE GOSSYPOL IN DIETS OF BREEDING CHICKENS. Poultry Sci. 31(5): 805-809. 1952.

Five selected groups of White Leghorn pullets were first fed diets containing approximately 0.026 percent free gossypol and later the same diet alone or supplemented with DL-lysine, glycine, 1-3 diamino propanol, or urea as sources of amino groups. Two other groups of the pullets were first fed diets containing 20 percent sardine meal. Later one group was fed the same diet supplemented with raw decorticated cottonseed as a source of approximately 0.026 percent free gossypol; the other was fed the unsupplemented diet. Data showed that the unfavorable effects of gossypol on yolk color and hatchability were not counteracted by the four compounds or the sardine meal.

129. BIRD, H. R.

COTTONSEED MEAL FOR POULTRY. Grain & Feed Jour. Consolidated 108(11): 38. 1952.

Cottonseed meal as a protein supplement in poultry rations has been limited due to a toxic pigment (gossypol) and a lysine deficiency.

Experimentally processed cottonseed meals were prepared at the Southern Regional Research Laboratory and South Texas Cotton Oil Co. Superior meals were obtained by employing mild conditions of processing.

The best meal prepared under the conditions in this experiment compared favorably with a commercial soybean oil meal when each was used as the sole protein supplement.

It has been found that cottonseed meals supplemented in rations for poultry are improved when fed in combination with either soybean oil meal or some other source of lysine, such as fish meal.

The presence of some toxic factor other than gossypol has been reported. Gossypol itself is toxic to chicks, depresses hatchability when fed as 0.016 percent of the diet, and causes egg yolk discoloration in diets containing 0.008 percent free gossypol.

In order to recommend unlimited standardized levels of cottonseed meal in poultry rations, more information

is needed on the effect of processing on the free gossypol content of meals.

130. GRAU, C. R.

USE OF COTTONSEED MEAL IN CHICKEN AND TURKEY NUTRITION. Calif. Animal Indus. Conf. Proc. 6: 55-56; Flour & Feed 54(7): 24. 1953.

The value of cottonseed meal as a source of amino acids, non-protein nutrients and its limitations in the nutrition of the chicken and the turkey are briefly discussed.

It is pointed out that while the cottonseed protein has been shown to contain a complete complement of all the amino acids necessary for the growing chick, it is only slightly above the minimum in lysine. This essential amino acid, whose availability in the meal is decreased by prolonged exposure to heat and moisture during processing, can be effectively supplemented by the addition of a lysine-rich protein concentrate. The limitations and usefulness of the practical ration based on grains and the sole protein source ration as methods for studying the feeding value of cottonseed meal are discussed. The latter is most useful for studying the completeness of a protein source and is also valuable for quickly testing the effect of processing variables on the nutritive value of a protein. Practical chick and turkey poult diets utilizing commercially produced meals at high levels of feeding was found to be successful and combining the meal with soybean oil meal was shown to give better results than when either meal was fed alone.

In a discussion of the limitations of cottonseed meal feeding, it was emphasized that while cottonseed meal containing 0.04 percent or less free gossypol can be fed in unrestricted amounts to chickens and turkeys and with no adverse effect on growth, for the present it is inadvisable to feed cottonseed meal to laying hens.

131. GRAU, C. R.

COTTONSEED MEAL IN POULTRY NUTRITION. Florida Poultry & Farm Jour. 20: 10, 15. Jan. 1954.

A general discussion is presented on the use of cottonseed meal in poultry and turkey rations.

The value of cottonseed as an amino acid source and as a nonprotein source and the limitations in using cottonseed in feeds is given, along with methods for evaluation of nutritive quality.

132. ALTSCHUL, A. M.

THE EFFECT OF PROCESSING CONDITIONS ON THE NUTRITIVE VALUE OF COTTONSEED MEAL FOR POULTRY--A REVIEW. Poultry Sci. 33(1): 180-185. 1954. Abstract in Assoc. South. Agr. Workers Proc. 50: 180. 1953.

Combined results of investigations on many types of cottonseed meals have indicated that meals suitable for unrestricted inclusion in poultry feeds can be prepared in commercial mills. Controlled experiments in several types of mills have shown that high cooking temperatures and/or high energy input to presses will lower the nutritive value of the meals. With meals containing 0.04 percent or less free gossypol, no interference was noted in the growth of chicks. However, egg-yolk discoloration was noted if high concentrations of cottonseed meals containing 0.03 percent free gossypol were present in the diets of laying hens.

An outgrowth of these experiments has been the formation of a cooperative cottonseed research program which has as its goal, the development of economic methods for regularly producing meals suitable for unrestricted use in poultry feeds.

133. MORGAN, C. L., and WILLIMON, C. P.

COTTONSEED MEAL PREPARED BY DIFFERENT METHODS FOR BROILER RATIONS. Poultry Sci. 33(3): 528-532. 1954.

Screw-press, solvent-extracted and hydraulic cottonseed meals in combination with soybean meal and supplemented with limited quantities of fish meal and dried whey, various vitamins, minerals, and methionine produced satisfactory gains in broilers to 10 weeks with a high efficiency of feed utilization.

Degossypolized solvent-extracted cottonseed meal as the sole protein supplement in the rations used gave growth rates which were not significantly less than those obtained with soybean meal but was not equal in feed efficiency.

The screw-press and hydraulic cottonseed meals used in this experiment when used alone as the protein supplement produced weights in broilers to 10 weeks that were significantly less than those on the soybean control ration.

134. HEYWANG, B. W., BIRD, H. R., and THURBER, F. H.
OBSERVATIONS ON TWO COMPONENTS OF
COTTONSEED THAT CAUSE DISCOLORATIONS IN
EGGS. Poultry Sci. 33(4): 763-767. 1954.

Laying White Leghorn pullets were fed diets containing cottonseed products for 40 days and all sound eggs examined for discolorations after varying periods of storage (1 to 6 months) at 36°F. The component responsible for pink albumen is present in raw cottonseed, crude cottonseed oil, and in cottonseed pigment glands, but not in cottonseed hulls. It was present in hexane-extracted cottonseed meal, but not in the meal which had been extracted with methyl-ethyl ketone or iso-butane. Six of the diets had a relatively high content of free gossypol and produced highly discolored egg yolks. Cottonseed hulls in the diet did not produce discolored yolks, and virtually no discoloration resulted when a gossypol-glycine complex was fed.

135. GRAU, C. R., ALLEN, E., NAGUMO, M., WORONICK, C. L., and ZWEIGART, P. A. COTTONSEED MEAL IN POULTRY FEED: A DISTINCTIVE YOLK COMPONENT IN THE FRESH EGGS OF HENS FED GOSSYPOL. Jour. Agr. and Food Chem. 2(19): 982-986. 1954.

Cottonseed meal is a desirable protein concentrate, but it is not now used in rations for laying hens because the gossypol it contains has an adverse effect on egg quality, particularly of stored eggs. In this study, an attempt was made to fractionate yolks of normal and "gossypol" eggs to discover differences between them. It was found that yolks of hens fed gossypol contain a yellow component not extractable by acetone, but soluble in 3:1 hexane-acetone. Although the amount of this component, estimated by its absorbance at 400 m μ , was related directly to the level of gossypol fed, its absorption spectrum was different from that of gossypol. Quantitative estimation of the egg yolk component will serve as a convenient measure of biologically active gossypol. This work is one step toward obtaining sufficient knowledge to allow use of cottonseed meal in diets of laying hens.

136. HEYWANG, B. W., and BIRD, H. R.
EGG PRODUCTION, DIET CONSUMPTION, AND LIVE
WEIGHT IN RELATION TO THE FREE GOSSYPOL
CONTENT OF THE DIET. Poultry Sci. 33(4): 851-
854. 1954.

Two feeding experiments were performed using groups of 25, laying White Leghorn pullets. In the first

experiment (180 days' duration) raw decorticated cottonseed was used to give free gossypol levels of about 0.008, 0.012, 0.016, 0.020, 0.025, and 0.033 percent. In the second experiment one diet contained the raw decorticated seed and the other contained solvent-extracted cottonseed meal; the levels of these materials being adjusted so that the free gossypol contents were about 0.008, 0.012, 0.016, and 0.020 percent. Both experiments clearly revealed that egg production and diet consumption were adversely affected by a free gossypol content of 0.020 percent or higher, while some adverse effects were obtained at 0.016 percent. The weight of the pullets was not adversely affected at 0.008, 0.012, 0.016, and 0.020 percent levels.

137. CURTIN, L. V.

USE OF LOW GOSSYPOL COTTONSEED MEALS IN SWINE AND POULTRY FEEDS. *Feedstuffs* 26(48): 49, 56-58, 60-2, 66. 1954.

Insofar as free gossypol is concerned, degossypolized cottonseed meals can be fed in unrestricted proportions in balanced rations for chicks, broilers, turkey poults, and swine.

Degossypolized cottonseed meal with a minimum of 75 percent soluble nitrogen can be used to replace 50 percent of the soybean oil meal in balanced rations for broilers and turkey poults with equal or improved results.

Degossypolized cottonseed meal with 44 percent protein and a minimum of 80 percent nitrogen solubility can be used to replace 80 percent of the soybean oil meal in balanced rations for broilers and turkey poults with no change in growth or feed efficiency.

Degossypolized cottonseed meal with a minimum of 75 percent soluble nitrogen can be used to supply 50 percent of the high protein supplement in balanced rations for growing-fattening swine.

138. WEST, J. W.

COTTONSEED MEAL USED IN POULTRY STARTER RATIONS. *Mississippi Agri. Expt. Sta. Inform. Sheet* 502, 2 p.; *Mississippi Farm Res.* 17(10): 1,8. 1954.

Degossypolized solvent-extracted cottonseed meal was used to replace 0, 20, 40, 60, 80 and 100 percent of the soybean oil meal in practical-type broiler and turkey starter rations.

Cottonseed meal of high quality may be used to replace as much as 80 percent of the soybean meal in broiler and turkey-starter rations without adverse effect upon growth and feed efficiency and can result in a saving of \$3-\$5 per ton of feed.

Complete substitution of soybean meal with cottonseed meal resulted in a reduced growth rate for broilers and poults and a reduced feed efficiency in most cases.

Combinations of cottonseed and soybean meals improved growth of chicks and poults as much as 5 percent over soybean meal fed singly.

Feeding of cottonseed meal did not appear to influence mortality, uniformity of broilers, carcass finish, or moisture content of the litter.

Generally, cottonseed meal is not recommended in laying rations, since extremely small quantities of gossypol have been found to cause yolk discoloration of eggs in cold storage.

Meals suitable for fowl may not be suitable for other animals, and processing history of the meal should be known prior to any feeding and preparations of diet mixtures.

139. HEYWANG, B. W.

SUITABILITY OF COTTONSEED MEAL IN DIETS FOR LAYING CHICKS. World's Poultry Congress, 10th. Sect. Papers No. 39, 3 p. 1954.

An unknown component of cottonseed causes pink albumen and pink to brown yolk discoloration in eggs. A known component, gossypol, causes olive green discoloration of egg yolks. Five percent of the eggs were discolored when the ration contained 0.001 percent free gossypol and 60 percent were discolored where it contained 0.008 percent free gossypol. Soybean and cottonseed meals at the 15 percent level in the ration were equal in so far as egg production, feed consumption, and body weight were concerned. Hatchability was lowered at an 0.024 percent gossypol level, but not at the 0.012 percent level.

When pure gossypol was added to a gossypol-free ration at the 0.012, 0.024, and 0.036 percent levels, hatchability was lowered at the 0.024 percent level when the ration was fed immediately after mixing. When fed 7 days after mixing, lowering of hatchability occurred only

at the 0.036 percent gossypol level. Evidently some of the gossypol was inactivated by storage.

140. HEYWANG, B. W., BIRD, H. R., and ALTSCHUL, A. M.
RELATIONSHIP BETWEEN DISCOLORATIONS IN
EGGS AND DIETARY FREE GOSSYPOL SUPPLIED
BY DIFFERENT COTTONSEED PRODUCTS. Poultry
Sci. 34(1): 81-90. 1955.

Groups of 10 to 12 laying White Leghorn pullets were fed all-mash diets with raw decorticated cottonseed, or screw-press, hydraulic, solvent-extracted, or pre-press-solvent extracted cottonseed meal as the source of free gossypol at dietary levels from 0.001 to 0.008 percent. Yolk and albumen colors were checked on fresh eggs and on eggs stored in the refrigerator for periods up to 6 months.

Both the percent of eggs with discolored yolks and the degree of yolk discoloration increased as the free-gossypol content of the diets increased and as the time of cold storage increased. The effects of gossypol on egg yolks were less severe with the solvent extracted meal. The content of the component that causes pink albumen was related to the free gossypol content of the raw cottonseed and screw-press and hydraulic cottonseed meals.

141. COUCH, J. R., CHANG, W. Y., and LYMAN, C. M.
THE EFFECT OF FREE GOSSYPOL ON CHICK
GROWTH. Poultry Sci. 34(1): 178-183. 1955.

The tolerance level of chicks for free gossypol was determined by feeding graded levels of gossypol in the form of pigment glands.

When the free gossypol content of the total ration was 0.06 percent or less no detrimental effects on growth rate, mortality or feed efficiency were found. This means that if cottonseed meal were used in amounts as high as 40 percent of the total ration, the free gossypol content of the meal would have to be greater than 0.15 percent before gossypol toxicity would appear. Very few commercial cottonseed meals contain as much as 0.15 percent free gossypol.

When the free gossypol content of the total ration was above 0.06 percent, growth rate progressively decreased with increasing amounts of gossypol, mortality increased and feed efficiency was poor.

Adding lysine to the rations increased the rate of growth at all levels of gossypol, but did not change the gossypol tolerance level as measured by growth rate, mortality, and feed efficiency.

Abstract: Chang, W. Y., Lyman, C. L., [i.e. C. M.] and Couch, J. R. Effect of Free Gossypol on Chick Growth, Poultry Sci. 33(5): 1048. 1954.

142. KRATZER, F. H., DAVIS, P. N., and MARSHALL, B. J. COTTONSEED MEAL IN RATIONS FOR STARTING POULTS, GROWING TURKEYS AND TURKEY BREEDER HENS. Poultry Sci. 34(2): 462-468. 1955.

In two battery experiments with poults, cottonseed meal could replace soybean oil meal in the ration to the extent of supplying half of the supplementary protein and still obtain satisfactory poult growth. A ration containing cottonseed meal alone as a source of supplementary protein was deficient in lysine but not methionine.

Poults fed rations containing 5 percent fish meal and the balance of the supplementary protein supplied by soybean oil meal or soybean oil meal and cottonseed meal in a ratio of 2:1 grew satisfactorily.

Cottonseed meal or cottonseed meal and soybean oil meal in a ratio of 2:1 caused reduced growth to four weeks of age and evidence of a lysine deficiency. The differences were not apparent at market age and only the males of the group receiving soybean oil meal and cottonseed meal in a ratio of 2:1 were significantly heavier than other groups. Both growth and feather pigmentation data indicate that the lysine requirement is much reduced after four weeks of age.

The hatchability of fertile turkey eggs was not reduced by 7.2 or 14.4 percent of cottonseed meal in the breeder ration, although pink albumen and olive yolks developed on storage of eggs from these hens.

143. WEST, J. W. COTTONSEED MEAL AS A SUBSTITUTE FOR SOY-BEAN OIL MEAL IN POULTRY RATIONS. Poultry Sci. 34(3): 547-553. 1955. Abstract: Poultry Sci. 33(5): 1088. 1954.

Degossypolized solvent extracted cottonseed meal was used at various levels to replace soybean oil meal in practical-type broiler and turkey starter rations. The findings may be summarized as follows:

1. Degossypolized cottonseed meal may be used to replace as much as 80 percent of the soybean oil meal in broiler and turkey starter rations without adverse effect upon growth or feed efficiency.

2. Complete substitution of soybean oil meal with cottonseed meal resulted in a highly significant reduction in the growth rate of starting turkeys. Similar results were obtained with broilers in at least half of the tests reported in this study.

3. Combinations of cottonseed meal and soybean oil meal improved growth of chicks and poults as much as four and five percent over either meal did singly, thus indicating the possibility of a complementary relationship between the two meals. Although the increases were only slight, the pattern was quite consistent.

4. From the limited observations reported, cottonseed meal did not appear to influence uniformity, carcass finish or moisture content of the litter.

144. GREEN, D. E., and STEPHENSON, E. L.

THE RELATION BETWEEN NITROGEN SOLUBILITY OF COTTONSEED PROTEIN IN DILUTE ALKALI AND ITS AMINO ACID ADEQUACY FOR CHICKS. (Abs.) Poultry Sci. 34(5): 1198. Sept. 1955.

Several samples of pre-press solvent cottonseed meal were prepared in a manner such that they possessed different nitrogen solubilities in a 0.02N NaOH solution. The meals were then incorporated into corn-cottonseed meal-type chick diets as the principal source of protein. These diets were supplemented with graded levels of DL-lysine-HCl, DL-methionine and DL-tryptophan. As the nitrogen solubility of cottonseed meal decreased, the amount of supplementary DL-lysine-HCl required for maximum growth increased, with 0.7 percent being required when only 22 percent of the nitrogen was soluble in dilute alkali. The optimum amount of supplementary DL-methionine appeared to be 0.05 percent in all cases. There was no increase in the need for this amino acid when the nitrogen solubility was reduced. There was no apparent tryptophan deficiency in any of the diets.

145. GRAU, C. R., LAU, T. L., and WORONICK, C. L.
COTTONSEED MEAL IN POULTRY FEED: INACTIVATION OF GOSSYPOL BY TREATMENT WITH PHLOROGLUCINOL. Jour. Agr. and Food Chem. 3(10): 864-865. 1955.

During conventional processing much of the gossypol of cottonseed becomes bound in an unavailable form. Not enough is bound, however, to eliminate the possibility of gossypol-caused discoloration of yolks of eggs laid by hens fed cottonseed meal. Cottonseed meal was treated by a modification of an analytical procedure that utilized phloroglucinol, hydrochloric acid, and ethyl alcohol as the reagents and diets containing the treated meal were fed to hens. A sensitive biological test detected no available gossypol in the meal preparation, as measured by the gossypol-cephalin content of eggs from hens fed the meal.

Gossypol in the meals was determined at the Southern Utilization Research Branch.

146. HEYWANG, B. W., and BIRD, H. R.
RELATIONSHIP BETWEEN THE WEIGHT OF CHICKS AND LEVELS OF DIETARY FREE GOSSYPOL SUPPLIED BY DIFFERENT COTTONSEED PRODUCTS. Poultry Sci. 34(6): 1239-1247. Nov. 1955.

A series of experiments to determine the relationship between the dietary level of free gossypol and the growth of young chicks was conducted.

New Hampshire and White Leghorn chicks were fed diets containing ground raw decorticated cottonseed, or screw-press, hydraulic, solvent-extracted, or pre-press solvent-extracted cottonseed meals, or pure gossypol. Dietary levels of free gossypol furnished by those sources varied from about 0.008 to 0.075 percent in different experiments.

Feeding of the diets was started when the chicks were 1 day old and ended when they were 6 weeks old, except that some of the diets containing pure gossypol were fed for five weeks only. Data on growth, diet consumption, and efficiency of diet utilization indicate that the free gossypol content should not be greater than 0.016 percent when fed to White Leghorn chicks or greater than 0.020 percent when fed to New Hampshire chicks.

Growth rate was not increased when diets containing 0.008 percent or less free gossypol were fed in one experiment. Growth was depressed when cottonseed meals were fed at high dietary levels in one experiment but free gossypol did not appear to be responsible for this depression.

Mortality was low in each experiment, amounting to about 1-1/2 percent of all chicks used.

The cottonseed meals and pure gossypol were supplied by the Southern Utilization Research Branch and assays of the gossypol content of the different sources were made there.

SEE ALSO: 4, 5, 11, 67, 94, 95, 99, 102, 110, 113.

VI-B. FEED FOR SWINE

147. STEPHENSON, E. L., and NEUMANN, A. L.
THE USE OF DETOXIFIED COTTONSEED MEAL IN
SWINE NUTRITION. Jour. Animal Sci. 9(4): 669.
1950.

This study compares the toxic principles of three differently processed cottonseed meals when used in rations for growing pigs. The cottonseed meals were fed in quantities calculated to be sufficient to supply the total amount of protein necessary to supplement an all-vegetable diet for swine. An additional six percent of fish meal was added to compensate for an animal protein factor or amino acid deficiency. Of the three meals tested, only one appeared to be nontoxic at the levels fed. This meal, an experimental product of the Southern Regional Research Laboratory, supported excellent growth and none of the pigs receiving this meal showed any toxic symptoms. The pigs receiving the other two meals died approximately six weeks after being placed on the experiment.

148. STEPHENSON, E. L., and NEUMANN, A. L.
THE USE OF DETOXIFIED COTTONSEED MEAL AS
PROTEIN SUPPLEMENT FOR GROWING PIGS.
Assoc. South. Agr. Workers Proc. 48: 73. 1951.

This experiment was designed to study the effect of feeding different cottonseed meals to weaned pigs. Cottonseed meal was fed at a level calculated to supply the total supplementary protein needed; an additional 6 percent fish meal was added to compensate for amino

acid or APF deficiencies. Of the 3 meals tested, 2 were very toxic, and the pigs died between 4 and 6 weeks. The third meal, an experimental product of S.R.R.L., was nontoxic, even when fed at levels as high as 43 percent of the total diet. Additional studies with the third nontoxic meal indicated that the addition of lysine would improve the meal, but methionine and APF were of little or no supplementary value.

149. CUNHA, T. J., SHAWVER, C. B., SEWELL, R. F., PEARSON, A. M., WALLACE, H. D., and GLASSCOCK, R. C.

OBSERVATIONS ON SUPPLEMENTING CORN-COTTONSEED MEAL RATIONS FOR GROWING AND FATTENING PIGS. Assoc. South. Agr. Workers Proc. 48: 73-74. 1951.

Supplementing corn-cottonseed meal rations for growing pigs with the animal protein factor (APF) was successful in preventing mortality and also produced high average daily weight gains. The use of lysine, methionine and B-vitamin supplementation was of no help or somewhat inferior to the same unsupplemented diet both from the standpoint of rate of gain and feed requirements per pound of gain.

Pertinent details of the pathological examinations of pigs not surviving diets containing high gossypol contents are given.

The expeller cottonseed meal was from Southern Utilization Research Branch and contained 0.024 percent free gossypol.

150. WALLACE, H. D., COMBS, G. E., and CUNHA, T. J. AMINO ACID, FERROUS SULFATE, FISH SOLUBLES, AND ANTIBIOTIC B₁₂ ADDITIONS TO COTTONSEED MEAL RATIONS FOR WEANLING PIGS. Assoc. South. Agr. Workers Proc. 49: 60. 1952.

A corn-cottonseed meal diet (cottonseed meal supplied by the Southern Utilization Research Branch containing 0.03 percent free gossypol) fed to weanling purebred Duroc pigs produced increased average daily weight gains when either 0.8 percent DL-lysine was added or when one-half of the cottonseed meal was replaced by soybean meal. Supplementation of the corn-cottonseed meal-soybean meal diet with 0.4 percent DL-lysine or 0.4 percent DL-lysine and 0.075 percent DL-methionine was ineffective in further increasing the weight gains.

A cottonseed oil meal diet (cottonseed oil meal supplied by the Buckeye Cotton Oil Company containing 0.025 percent free gossypol) fed to purebred Duroc and Hampshire pigs did not produce an increased average daily weight gain when supplemented with Lederle Aurofac (Aureomycin plus B₁₂), fish solubles or ferrous sulfate.

151. STEPHENSON, E. L., NOLAND, P. R., and CAMP, A. A. COTTONSEED MEAL IN SWINE NUTRITION. Arkansas Agr. Expt. Sta. Bull. 523, 18 p. 1952.

Results are reported on a study of means of utilizing cottonseed meal in growing-fattening feeds for swine. The extreme toxicity of hydraulic or solvent-processed cottonseed meal was verified and substitution of soybean meal for one-half of the hydraulic meal only delayed the onset of the gossypol poisoning. A screw-pressed meal prepared by the Southern Utilization Research Branch was found to be a satisfactory protein supplement when fed at the 43 percent level of the total diet and the inclusion of 6 percent fish meal resulted in a protein supplement that was nearly as adequate for pigs as one composed entirely of fish meal.

The addition of an aureomycin-vitamin B₁₂ concentrate or 0.3 percent DL-lysine to the screw-pressed cottonseed meal diet did not consistently increase the rate of pig gain for the former supplement and was ineffective with the latter. The addition of 0.3 percent DL-lysine to the nontoxic cottonseed meal ration appreciably improved the rate of gain, indicating that the meal was probably low in lysine.

An ethanol extract of condensed fish solubles added some factor(s) not present in the basal diet of corn and screw-pressed cottonseed meal nor contained in a lysine or aureomycin-vitamin B₁₂ concentrate. A corn-soybean meal diet serving as positive control was appreciably improved by the addition of 2 percent of the ethanol extract of condensed fish solubles.

152. WALLACE, H. D., COMBS, G. E., and CUNHA, T. J. SUPPLEMENTS TO LOW-GOSSYPOL COTTONSEED MEAL RATIONS FOR WEANLING PIGS FED IN DRY LOT. Assoc. South. Agr. Workers Proc. 50: 66. 1953.

Experiments were conducted in two separate trials with weanling pigs fed in dry lot to determine the

feasibility of supplementing a ration composed of corn and a low-gossypol cottonseed meal (cottonseed meal supplied by the Southern Utilization Research Branch containing 0.015 percent free and 0.508 percent total gossypol) and adequate with respect to minerals and vitamins. A corn-soybean oil meal served as positive control.

The performance of all lots were considerably better in the first lot than in the second but were fairly complementary. The unsupplemented cottonseed meal ration was inferior to the control soybean oil meal ration, and iron supplementation was ineffective in the first trial but markedly improved weight gains were observed in the second; the surfactant and copper sulfate were ineffective in both trials. A high level of Aurofac effectively stimulated weight gains in each of the trials.

153. SINGLETARY, C. B., WATTS, A. B., CROWN, R. M., and DAMON, R. A.
COTTONSEED MEAL IN PRACTICAL RATIONS FOR GROWING AND FATTENING SWINE. Jour. Animal Sci. 12: 932. 1953.

A study was conducted to evaluate the quality protein in differently processed cottonseed meals when fed singly and in combination with solvent-extracted soybean oil meal. Practical swine rations were used in which cottonseed meal replaced soybean oil meal on an equivalent protein basis. The pigs were grown to weights of 200 pounds at which time they were slaughtered and carcass information was obtained.

In general, there were no significant differences obtained on pigs fed the various meals. Results indicate that 100 percent of standard vegetable protein can be replaced by low-gossypol cottonseed meals with satisfactory results.

154. GODBEY, E. G., and STARKEY, L. V.
LOW GOSSYPOL COTTONSEED MEAL AND ANTIBIOTICS IN SWINE RATION. Assoc. South. Agr. Workers Proc. 51: 68-69. 1954.

Eighty-five pigs averaging 80 pounds in weight were fed until a final weight of 200 pounds was reached. Self-feeders were used and the pigs were fed in individual pens on concrete floors. Poland-China, Duroc-Jersey, Hampshire, Berksshire, and Landrace pigs were used.

Ground yellow corn was fed in each lot. The supplements used were as follows:

Lot 1 (check) - 1/2 fish meal, 1/4 alfalfa, and 1/4 cottonseed meal.

Lot 2 - Alfalfa meal and low gossypol cottonseed meal, low temperature processed at SRRL.

Lot 3 - Alfalfa meal and low gossypol cottonseed meal, Buckeye Cottonseed Oil Co.

Lot 4 - Alfalfa meal and solvent extracted soybean oil meal.

Lot 5 - Lot 2 with Aurofac (Lederle) added.

Lot 6 - Lot 2 with MK 45 (Merck) added.

The antibiotic supplements used in Lots 5 and 6 were fed in such amounts that 18,000 micrograms of B₁₂ were added to each ton of feed. 18,000 milligrams of Aureomycin were added to each ton of feed by the Aurofac and 21,600 milligrams of Penicillin by the MK 45. 1 percent of ground limestone and 1/2 percent of salt was added to each ration.

The pigs in the check lot, where fish meal was used, made significantly faster gains than those fed in the vegetable supplement lots. When the antibiotics and B₁₂ were added to the vegetable supplement, the daily gains were increased to such an extent that they were not different from those made by the hogs in the check lot. The hogs in the low temperature cottonseed meal required more feed per pound gain than those fed in the check lot. When the antibiotics and B₁₂ were added to the low temperature cottonseed meal ration, the daily gains of the hogs were increased significantly and the feed per pound gain was reduced by a significant amount. The gains in feed per 100-pound gain were not significantly different for the two groups of hogs fed antibiotics.

155. WALLACE, H. D., McKIGNEY, J., and GILLESPIE, L. CORN-COTTONSEED MEAL RATIONS FOR WEANLING PIGS FED IN DRYLOT. Assoc. South. Agr. Workers Proc. 51: 69. 1954.

In view of the recent studies indicating that 14 percent of protein is adequate for weanling pigs in drylot fed well fortified corn-soybean meal rations, a study was designed to test the efficiency of such a level of protein using cottonseed meal low in gossypol (0.04 percent free gossypol). In addition a product known as DSC (distillers solubles concentrate) containing approximately 50 percent total protein and significant quantities

of lysine was tested for its supplementary value. A high level of Aureomycin (60 gms. per ton of feed) was also studied for its supplementary value. All rations were fortified with Aureomycin, Lederle Fortafeed 2.49 C, and minerals including trace minerals. Pigs were self fed on concrete floors.

Two experiments were conducted as duplicates covering a period of nine weeks following weaning and using two prepressed-solvent extracted cottonseed meals from different plants.

Results obtained from the two experiments on 6 lots indicate that supplementation of the 14 percent corn-cottonseed meal diets with 5 percent DSC decreased rather than improved average daily gains and feed efficiency, while the addition of 5 percent DSC together with Aureomycin at high levels gave some slight increases in weight gain and feed efficiency. The 20 percent corn-cottonseed meal rations DSC produced lower weight gains and feed efficiency than did the 14 percent corn-cottonseed meal DSC diets.

The corn-soybean meal rations, with or without DSC, proved to be slightly superior to those of corn-cottonseed meal.

156. WALLACE, H. D., McKIGNEY, J., and GILLESPIE, L. PREPRESSED SOLVENT EXTRACTED COTTONSEED MEAL AS A SOURCE OF PROTEIN FOR THE WEANLING PIG. Florida Agr. Expt. Sta. Animal Husbandry Mimeo. Ser. 7, 5 p. 1954.

Nine-week drylot feeding tests employing Duroc and Duroc x Hampshire weanling pigs have indicated that solvent-extracted soybean oil meal was a more satisfactory protein supplement than prepressed cottonseed meal (0.04 percent "free" gossypol). Addition of a distillers solubles concentrate (a by-product of the production of monosodium glutamate from wheat gluten) or a high level of Aureomycin failed to improve growth of pigs fed on the cottonseed meal diets.

The first experiment was complicated by dermatitis but in the second experiment a 20 percent protein cottonseed meal ration promoted faster gains than did a 14 percent ration. The dermatitis syndrome is not peculiar to corn-cottonseed meal rations alone, since it has been observed many times on corn-peanut meal and corn-sunflower seed meal rations.

157. MINER, J. J., CLOWER, W. B., NOLAND, P. R., and STEPHENSON, E. L.

AMINO ACID SUPPLEMENTATION OF A CORN-COTTONSEED MEAL DIET FOR GROWING-FATTENING SWINE. Jour. Animal Sci. 14(1): 24-29. 1955.

A significant growth response was obtained by supplementing a corn-cottonseed meal type diet for swine with DL-lysine. There was a highly significant difference in the response resulting from feeding different levels of DL-lysine. The optimum level of supplementary DL-lysine fed in the presence of fish solubles was 0.1 percent. Levels of supplementary DL-lysine greater than 0.1 percent appeared to depress growth, whereas, levels lower than 0.1 percent failed to support maximum growth.

The addition of DL-tryptophan at levels used in these experiments promoted growth significantly when added in the presence of fish solubles.

The addition of 0.05 percent DL-methionine did not result in a statistically significant growth response.

Significant increases in growth rate were paralleled by increases in efficiency of feed utilization.

158. WALLACE, H. D., CUNHA, T. J., and COMBS, G. E.
LOW-GOSSYPOL COTTONSEED MEAL AS A SOURCE OF PROTEIN FOR SWINE. Florida Agr. Expt. Sta. Bull. 566: 26 pp. 1955.

A 4-year study (1950-54) involving 286 weanling pigs fed in dry lot and 24 brood sows fed on pasture during gestation and lactation was conducted to determine the value of low-gossypol cottonseed meal in swine rations.

Specially, processed low-gossypol meal containing only 0.024 percent of free gossypol was tolerated by the weanling pigs in quantities up to 35 percent of the ration. Subsequent experiments suggested that 0.04 percent of free gossypol was a safe upper limit for cottonseed meal used in swine feeding.

When low gossypol meal was the only supplemental protein, the performance of the pigs was less than optimum.

The effect of several supplemental feed materials added to a ration compounded of yellow corn and low-

gossypol cottonseed meal was studied. Neither a vitamin mixture nor a high level of Vitamin A and thiamine supplementation proved effective.

A high level of Aureomycin was generally beneficial. However, the response to such supplementation was variable and appeared to be influenced by other factors in addition to the ration itself.

Iron sulfate (0.25 percent), fed in two experiments, appeared to be beneficial in four of six comparisons.

Enormous individual variation in growth among animals fed cottonseed meal rations were not observed when the protein supplement was soybean meal. The last series of growing-fattening experiments showed a 50-50 combination of low-gossypol cottonseed meal and soybean meal was superior to soybean meal alone.

Results also indicated that the two meals were equally valuable for sows during gestation and lactation when fed on pasture.

159. HOLLEY, K. T., HARMS, W. S., STORHERR, R. W., and GRAY, S. W.

COTTONSEED MEAL IN SWINE AND RABBIT RATIONS. Georgia Agr. Expt. Sta. Mimeo. Ser. N. S. 12: 27 p. 1955.

In repeated dry-lot tests reported here pigs on rations containing 0.012 percent free gossypol, by the phloroglucinol method, developed the symptoms of cottonseed meal toxicity and losses were heavy. In this same series of tests, pigs on rations containing 0.006 percent free gossypol developed no symptoms of cottonseed meal toxicity and made satisfactory daily gains under fair feed efficiency rates. Under these conditions then the critical level of free gossypol in cottonseed meal for pigs is between 0.06 and 0.03 percent. These values are not far above and below the 0.04 percent free gossypol limit proposed as a tentative safe upper level in cottonseed meal by the third conference at SRRL. (See Item 3).

Apparently rabbits are more sensitive to cottonseed meal toxicity than pigs, since deaths occurred in shorter feeding periods, and some losses at a level of 0.04 percent free gossypol in the meal.

SEE ALSO: 4, 5, 11, 14, 137.

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